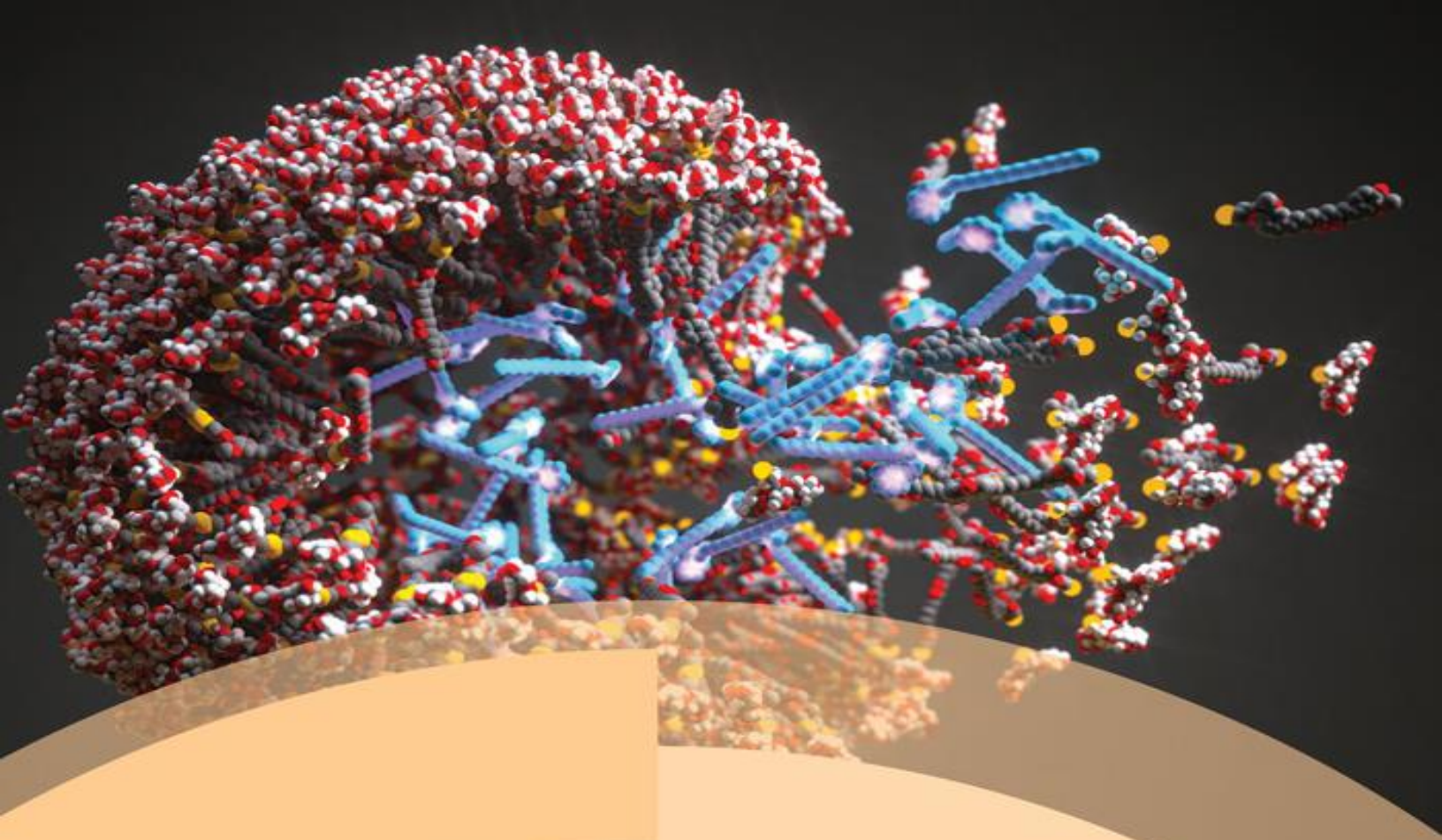




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Polysaccharide-based Biomaterials

Delivery of Therapeutics and Biomedical Applications

Edited by Sougata Jana, Subrata Jana and Abraham J. Domb

Polysaccharide-based Biomaterials

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Preface

Polysaccharides are important natural, renewable biomaterials that are biodegradable and biocompatible in nature. Polysaccharide-based biomaterials are widely used for delivery of therapeutics in biomedical fields. This book focuses on the main polysaccharides, including but not limited to chitosan, cellulose, alginate, dextran, guar gum, gellan gum, pullulan, locust bean gum, pectin, xanthan gum, starch, hyaluronan, and carrageenan, and their versatile applications in drug delivery, biomedical imaging, and tissue engineering.

This book contains eighteen chapters describing systematic development to present scenarios in the field for the widely used polysaccharide-based biomaterials.

The book starts with an introduction chapter (Chapter 1) containing a general overview of polysaccharide-based biomaterials.

Chapter 2 discusses recent advances in alginate polysaccharide-based carriers for the delivery of therapeutics and biomedicine, whereas Chapter 3 focuses particularly on the development of alginate-based carriers for transdermal drug delivery systems (TDDSs). TDDSs have been an efficient approach for the pharmaceutical and cosmetic industries in recent years. Several protocols for transdermal delivery are being used in drug targeting, including microneedle-based formulations, transdermal patches, and gels, which are outlined in this chapter.

Polysaccharide-based nanocarriers are widely used to enhance the therapeutic efficacy of drugs and other pharmacologically active agents. Chapter 4 is framed on chitosan-based nanocarriers for drug delivery. Chitosan is usually obtained by the deacetylation of chitin, a naturally abundant polysaccharide mainly found in the exoskeleton of crustaceans and insects. It has attractive properties such as mucoadhesiveness, biocompatibility, biodegradability and non-toxicity, and antimicrobial activity. Current challenges and future perspectives on the real use of this chitosan-based nanosystem are also presented.

Chapter 5 highlights hyaluronic acid (HA) and its versatile applications in drug delivery, gene delivery, and imaging systems. HA is a natural biodegradable and biocompatible polysaccharide that can play a significant role as a carrier for the delivery and controlled release of different biomolecules.

Chapter 6 covers dextran-based nano-carriers and their application in drug delivery, with special emphasis on its preclinical and clinical studies. Dextran is a non-toxic, complex, biodegradable, and non-immunogenic polysaccharide.

Chapter 7 describes guar gum-based carriers for controlled release and delivery of drugs and their therapeutic applications.

Chapter 8 explores gellan gum derivatives and gellan gum-based drug delivery systems. Gellan gum is an exopolysaccharide that has been widely used in pharmaceutical and biomedical fields due to its different unique physicochemical and biological properties.

In recent years locust bean gum (LBG) has emerged as a versatile polysaccharide with various pharmaceutical applications. Chapter 9 discusses the manufacturing methods, physicochemical properties, and pharmaceutical uses of LBG, particularly in several drug delivery systems.

Pectin is a plant-derived, edible heteropolysaccharide with an excellent biocompatible and biodegradable nature. Various administration routes of delivery systems for the delivery of therapeutic agents such as oral, intravenous, intranasal, topical, ocular, and rectal are highlighted in this chapter. Chapter 10 focuses on pectin-based carrier systems for the delivery of therapeutics and biomedical applications.

Pullulan is an important non-toxic, biodegradable, and biocompatible polysaccharide easily obtained from fungal fermentation. Chapter 11 describes pullulan and its derivatives as drug carrier systems. The therapeutic activities of pullulan such as antioxidant, anti-inflammatory, immunomodulatory, antitumor and anti-glycemic, anti-lipidemic, antibacterial and antifungal, etc. are also thoroughly discussed.

Carrageenan is a naturally sulfated polysaccharide obtained from marine red seaweeds. It is safe, non-toxic, biodegradable, and biocompatible, with anticoagulant and antitumor qualities. Chapter 12 explores carrageenan-based carrier systems (nano/micro particles, pellets, hydrogels, wafers, beads, microspheres, and suppositories) for therapeutic delivery.

Chapter 13 offers a brief overview about the history, production, chemical composition, physico-chemical properties, and different formulations of xanthan gum and its derivatives. The potential use of xanthan gum and its derivatives in designing formulations such as nanoparticles, microparticles, tablets, hydrogels, and complex matrices are summarized along with their applications in drug delivery.

Chapter 14 presents recent applications of cellulose-based materials as drug delivery systems.

Chapter 15 describes the various approaches and their mechanisms involved in the fabrication of starch-based drug delivery systems for biomedical applications. Starch is the most abundant renewable biopolymer and is widely used as a promising candidate for drug delivery and biomedical applications as a binder, filler, and disintegrant due to its superior loading efficiency and controlled release of drugs, therapeutics, enzymes, and bioactive compounds to the targeted site.

Tamarind seed polysaccharide is widely utilized as a mucoadhesive biopolymer and *in-situ* gelling agent through various routes. Its most common usage is as a release retardant in tablets or multiparticulate systems. Chapter 16 focuses on tamarind seed polysaccharide and its modified form in drug delivery, wound dressing, tissue regeneration, and engineering applications.

Chapter 17 focuses on chitosan-based biomaterials for anti-cancer drug delivery and biomedical imaging. This chapter emphasizes the synthesis of formulation-based nanoparticles, microparticles, nanocomposites, hydrogels, and conjugates of chitosan for cancer theranostics.

The last chapter, Chapter 18, discusses a recent update on polysaccharide-based scaffold materials in tissue engineering, such as in different fields of bone regeneration, cartilage regeneration, skin regeneration, cardiac regeneration, and neural regeneration.

In summary, we believe that the specially selected topics on polysaccharide-based biomaterials with a special emphasis on the delivery of therapeutics and biomedical applications will be useful for readers. This book is an important resource for academics and researchers working in the field of polysaccharide-based biomaterials for drug delivery under material science, chemical science, pharmaceuticals, life science, and biotechnology as well as biomedical and other health care professionals.

We acknowledge the contribution from all the authors with sincere gratitude. This book would not have been possible without their support.

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Chapter 1

Polysaccharide-based Biomaterials: Overview

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1.1 Introduction

The use of natural biomaterials for biomedical and tissue engineering applications has been steadily increasing due to their non-toxic nature, biocompatibility, biodegradability, and cyto-compatibility without eliciting an immune response.¹ Chemically, polysaccharides are the macromolecules composed of monosaccharide units joined covalently by *o*-glycosidic linkages. The polysaccharide structure offers the availability of hydroxyl, carboxylic, and amine functionalities as potential sites for possible structural modifications which assist to tune physicochemical properties by chemical modifications. These have been recognized as green materials

derived from natural resources. They are, hence, employed as green alternatives for biomedical applications.² These polysaccharides can be easily isolated from renewable sources such as animals, plants, and microorganisms. Usually, synthetic polymers lack the desired biocompatibility and bioactivity and may exhibit toxicity, inflammation, and low biological activity. However, these biomaterials exhibit several advantages, such as natural abundance, ease of isolation, and opportunity for structural modifications as needed. This has been reflected in a continuously increasing number of publications in this area. Easy modification in the properties of these materials is possible due to the presence of multiple functionalities on the polymer backbone.³ The natural polysaccharides of plant and animal kingdom origin are useful in biomedical applications, including drug delivery and tissue regeneration. Most of the natural biomaterials are present in the extracellular matrix in living systems and are involved in inter- and intra-cellular cell signaling contributing to cell growth. These materials have several physiological functions, such as promoting the formation of cytokines stimulation of macrophages and lymphocytes, and promotion of antibody production to enhance organismal immunity. Consequently, polysaccharide-based biomaterials are easily recognized and widely accepted by the human body. These biomaterials undergo easy enzymatic or hydrolytic degradation in biological environments and exhibit several advantages such as natural abundance, ease of isolation, possibilities of structural modifications, and enzymatic/hydrolytic biodegradation.⁴ Although these materials suffer from microbial contamination, uncontrolled water uptake, poor mechanical strength, as well as unpredictable degradation patterns, advancements in material science and nanotechnology assist to overcome these drawbacks by structural modifications. Research encompasses the development of new materials with enhanced mechanical stability, degradation, and bioactivity, making them useful as biomaterials in the form of films, fibers, 3D structures, and hydrogel in drug delivery, tissue regeneration, and other biomedical applications. These diverse biopolymers exhibit a wide molecular weight range along with the presence of charges and monomer compositions, contributing to diverse properties and biomedical applications. Each of these biopolymers has varied biocompatibility,

biodegradation ability, hydrophilicity, water solubility, and biological activities. Numerous structural modifications allow the articulation of biomaterials with desirable properties and applications.⁵ Among the natural biopolymers, cellulose, chitosan, hyaluronic acid, alginates, heparin, dextran, pullulan, and chondroitin sulfate constitute the major sources of biomaterials useful in biomedical and tissue engineering applications. This chapter provides an overview of various polysaccharides and their potential applications as effective biomaterials and a future perspective in this field.

1.2 Cellulose

Cellulose, with the formula $(C_6H_{10}O_5)_n$, is the most abundant natural polysaccharide with tunable properties and has remained a widely used material for the development of various biomaterials in drug delivery and tissue engineering.⁶ Chemically, it is a carbohydrate comprised of glucose units attached by β -glycosidic linkages comprising an organized fibrillar structure. Unbranched chains of glucose are connected *via* β -1–4 glycosidic bonds (Figure 1.1).

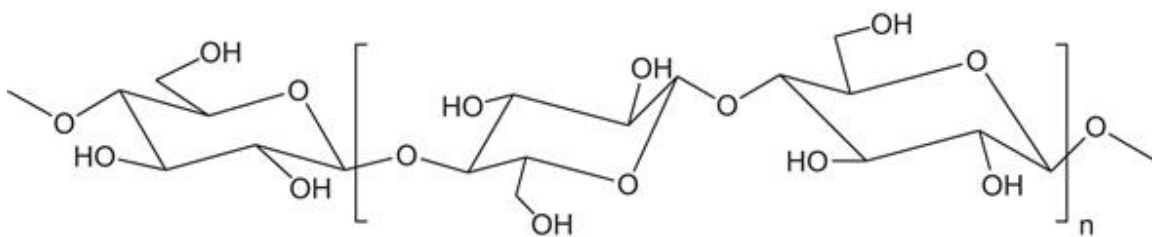


Figure 1.1 Chemical structure of cellulose.

Chains of the polysaccharide are aligned in parallel to form microfibrils. It is a tasteless and odorless material mainly present in the cell walls of plants, fungi, algae, and bacteria.⁷ Leafy green vegetables such as green peas, brussel sprouts, kale, *etc.*, are rich sources of cellulose.⁸

Cellulose with different compositions and morphology is available depending upon the source of origin. The presence of inter- and intra-molecular hydrogen bonding affords rigidity to the chain. The compositional and structural features of the materials do not allow it to

dissolve in common organic solvents. However, structural modifications assist to modulate the properties and articulate the engineered materials with desirable properties. Cellulose-based biomaterials, being environmentally friendly, find extensive applications in drug delivery; wound healing, as tablet binders, coating materials, and viscosity modifiers.⁹

Cellulose can be widely employed as a material for cartilage tissue engineering applications.¹⁰

Bacterial cellulose produced by aerobic bacteria possesses several advantages such as high purity, good porosity, strong water-absorbing capacity, and high mechanical strength along with good biocompatibility.¹¹

Among natural polysaccharides, cellulose has emerged as the most exploitable functional material in the nanoscale. It has several advantages and provides a platform for the growth and development of cells.¹²

Apple-derived cellulose scaffolds were analyzed and proved to possess good mechanical properties useful for bone tissue engineering applications. Studies showed that pre-osteoblasts can proliferate and adhere within the apple-derived cellulose constructs.¹³

Research work by B. N. Singh and K. Pramanik reported the development of bioactive nanocomposite scaffolds of nanobioglass/silk fibroin/carboxymethyl cellulose. The researchers determined them to be applicable for bone tissue engineering. Electrospun nanofibrous composites of carboxymethyl cellulose (CMC), silk fibroin (SF), and nano-bioglass (nBG) mimicking the native bone extracellular matrix were developed using a free liquid surface electrospinning technique. *In vitro* culture of hMSCs using the scaffolds support the proliferation, adhesion, and viability of cells. The developed scaffold possesses good physicochemical and biological properties, making it suitable for the osteogenic differentiation and growth of hMSCs, constituting a suitable material for bone tissue applications.¹⁴

Among the various materials of cellulose, cotton cellulose is easily available and is lignin free and FDA approved. It can be a derived scaffold by using cotton microfibers for bone tissue engineering applications. Attempts were made to improve the osteogenic, physicochemical, and mechanical properties of the scaffolds by crosslinking with citric acid

followed by modifying with gelatin. Citric acid can effectively crosslink the cellulose microfibers, providing porosity and a swelling ability, resulting in an increase in the water absorption capacity and compressive strength of the scaffold.¹⁵

The biocompatibility of bacterial cellulose can be assessed in the corneal stroma replacement scaffold. The studies carried out on rabbit stromal and corneal epithelial cells cultured on the BC scaffold reveal growth of primary cells. The biocompatibility of the material after a period of 3 months reveals growth of epithelial and stromal cells at a survival rate of nearly 100%, supporting good biocompatibility of the bacterial cellulose as an excellent material for the tissue engineering of corneal stroma.¹⁶

A bilayer matrix can be fabricated by using hybrid cellulose acetate nanofibers (CA) containing bioactive latex or ciprofloxacin over a collagen (CSPG) 3D matrix by using an electro spinning technique.

The bilayer matrix exhibits a 3D porous and nanofibrous structure possessing high stability, porosity and a good swelling ability which makes them suitable in soft tissue for wound healing applications. The bioactive latex present in the bilayered matrix functions as a drug inhibiting the infection at the wound site along with good cell adhesion and non-toxicity among the *in vitro* biological and fluorescence properties.¹⁷

Bacterial cellulose membranes serve as biocompatible biomaterials for mesenchymal and induced pluripotent stem cell culture and for tissue engineering applications.¹⁸

The extracellular matrix (ECM) provides structural support for cell growth. It also helps in the attachment and proliferation greatly affecting the cell structure and functioning. Marine macroalgae species *Ulva* sp. and *Cladophora* sp. can be evaluated as alternative ECM materials. B.-S. Nurit *et al.* used the decellularization–recellularization approach and fabricated seaweed cellulose-based scaffolds for *in vitro* mammalian cell growth. The scaffolds were proved to be nontoxic.¹⁹

3D-Printed bone scaffolds constitute promising materials for the treatment of critical-sized bone defects. Although poly(+ -caprolactone) (PCL) is an important material useful to treat bone defects, it possesses relatively low stiffness and lacks bioactivity. This can be overcome by the use of carboxyl group decorated surface-oxidized cellulose nanocrystals

(SO-CNCs) to improve the mechanical properties of PCL and result in the induction of biomineral formation upon PCL.²⁰

Wood-based cellulose nanofibrils (CNFs) possess high surface reactivity and the ability to mimic nanostructured collagen in the bone extracellular matrix. Cross-linked gelatin–CNF hydrogels serve as promising materials for BTE. The cross-linking and modified mechanical properties of the cellulose do not produce any adverse biological responses to the bones that support osteogenic differentiation.²¹

Bacterial cellulose (BC) can be used for tissue regeneration and regenerative medicine. BC mats can be used in treating affected skin such as ulcers and burns. The fibrous morphology of the biopolymers supports cell proliferation. Researchers have found that the bacterial cellulose fermentation process can be modified by adding vegetal stem cells to the natural materials and culture medium.²²

Carboxymethyl cellulose (CMC) is an anionic, biocompatible, biodegradable, and water-soluble biopolymer possessing excellent hydrophilicity, chelating, and antifouling potential. CMC hydrogels have been extensively employed as smart polyelectrolytes due to pH sensitivity, swelling ability, and variations in ionic strength variations. It has been used in drug and protein delivery as well as pharmaceutical applications. CMC scaffolds cross-linked with citric acid can be processed by the freeze-drying method. Their osteogenic and osteoinductive abilities have been proven.²³

Methacrylated gelatin (GelMA)/bacterial cellulose (BC) composite hydrogels are developed by immersing BC particles into GelMA solution and then subsequently photo-crosslinking. Such composite hydrogels exhibit interconnected porous network structures and show improved mechanical properties compared with the pure GelMA. These GelMA/BC composite hydrogels can be used for cartilage tissue engineering. The GelMA/BC hydrogels support chondrocyte ingrowth, proliferation, and phenotype maintenance.²⁴

Cellulose-based hydrogels possess great potential for application in tissue engineering. The hydrogels can be prepared from native cellulose (both bacterial as well as plant sources) or cellulose derivatives, viz. methylcellulose, hydroxypropylmethylcellulose, and carboxymethylcellulose. Cellulose–polymer composites are highly useful

and attractive, inexpensive, and advantageous structural materials. These are useful in several tissues such as cartilage, blood vessels, the heart, and bones.²⁵

Methylcellulose (MC) is another attractive material useful for the preparation of thermo-responsive hydrogels. These hydrogels undergo sol–gel transition and show physicochemical and mechanical responses to temperature changes. MC-based responsive systems can be easily prepared and constitute promising results in both *in vitro* and *in vivo* studies.²⁶

The biological heart valve prostheses that are currently-used may show drawbacks in the long-term. E. Chainoglou and co-workers have developed cellulose-acetate-based nanoscaffolds. The researchers have used them as coverings on aortic heart valve surfaces to create an artificial valve. Such artificial valves can stimulate the properties of a native valve. The presence of bioactive factors like laminins and RGD peptides makes the surface of the valve more biocompatible and provides controlled endothelialization of cardiac valves, causing an anti-coagulant effect and therefore a reduction in valve thrombosis.²⁷ Table 1.1 summarizes recent cellulose-based biomaterials for biomedical applications.

Table 1.1 Recent biomedical applications of cellulose-based biomaterials.

S. no	Biomaterial	Application	Ref.
1	Nano composite scaffolds of nanobioglass/silk fibroin/carboxymethyl cellulose	Bone tissue construct	14
2	Cross-linked gelatin–CNF hydrogel	Bone tissue engineering	21
3	Bacterial cellulose (BC)	Treatment of affected skin such as ulcers and burns	22
4	Carboxymethyl cellulose (CMC) cross-linked with citric acid	Drug/protein delivery	23
5	Methacrylated gelatin (GelMA)/bacterial cellulose (BC) composite hydrogels	Cartilage tissue engineering	24

1.3 Chitosan

Chitosan is the second most abundant natural polysaccharide biomaterial. Chemically, it is composed of β -(1 $\rightarrow$ 4)-2-amino-2-deoxy- α -glucose and is a product of partially or fully deacetylated chitin (Figure 1.2). It is soluble in water as well as in many organic solvents. It offers several sites and opportunities for chemical modifications.

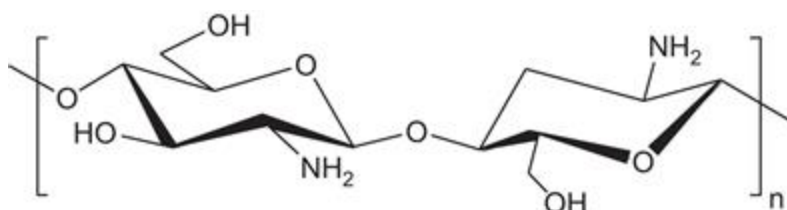


Figure 1.2 Chemical structure of chitosan.

Most of the characteristic properties of chitosan are mainly due to the presence of a primary amine group found along its backbone. Chitosan can be modified by grafting pendant -NH_2 or -OH functional groups without modifications in the polymer chain and hence constitutes a popular material for biomedical applications. This natural biomaterial exhibits unique biocompatibility, biodegradability, and an affinity for biomolecules and has become popular for biomedical applications.^{28,29}

Chitosan can be fabricated in the form of scaffolds, gel, 2D/3D films, and fibers as well composites with inorganic matter.³⁰

Chitosan-based covers different materials used in drug/gene delivery systems, wound healing, hemodialysis membranes, implant coatings, bone and tissue regeneration/engineering, and other bioactive entities used as biomaterials for different therapies. A wide range of biomedical applications of chitosan-based biomaterials is on account of a biocompatibility, biodegradable nature without the formation of acidic degradation products, and non-toxicity.³¹

Chitosan has antibacterial, non-toxic, and wound healing properties. It has a wide affinity for *in vivo* macromolecules and is, hence, useful in cell ingrowth and osteoconduction applications. These peculiar properties of materials make chitosan-based materials a promising platform to develop improved bio-distribution, increased specificity, and sensitivity to afford various materials for biomedical applications. Many researchers are engaged in the fabrication and articulation of biocompatible and biodegradable biomaterials.³²

1.4 Modifications

Different derivatives of chitosan have been developed for potential biomedical applications. The structural modifications do not induce appreciable changes in the chitosan's fundamental backbone but improve the properties of the material. These chitosan derivatives are reported for their increase in the penetration of drugs, particularly for hydrophilic macromolecules, protecting acid-sensitive drugs, attaining colon targeting, and improving drug release capacity in the basic environment. The modifications can also overcome the restricted solubility of the chitosan in neutral pH. A variety of chemically-modified derivatives of chitosan can be synthesized through modification in the chitosan hydroxyl or amine functional groups. Also, modifications through thiolated, hydrophobic, quaternized, and chemically grafted chitosan derivatives are documented in the literature. *N*-Alkylated chitosan derivatives such as trimethyl, triethyl, diethyl methyl, and dimethyl ethyl chitosans are typically prepared *via* alkylation of the primary -NH_2 groups of chitosan with aldehydes in the presence of reducing agents. *N*-Trimethyl chitosan (TMC) can be synthesized by methylating amine groups of chitosan using methyl iodide. Also, the preparation of mono-*N*-carboxymethyl chitosan (MCC) can be accomplished by modifying -NH_2 functional groups of chitosan with glyoxylic acid in the presence of sodium borohydride. The MCC is compatible with anionic agents and suitable for promising applications in tissue culture.³³

The derivatives of quaternary ammonium chitosan such as *N*-(2-hydroxyl)propyl-3-trimethyl ammonium chitosan chloride (HTCC) can be

developed by introducing a quaternary ammonium moiety into the chitosan amine groups. The hydrophobic chitosans increase the stability of the scaffold due to a decrease in matrix hydration. Hence, they show elevated resistance to the gastric enzymatic degradation.³⁴

The hydrophobic groups of chitosan can be acylated by using fatty acid chlorides, sodium salts of fatty acids, and the use of lactones as a polymeric matrix for drug delivery applications. Caprylic, lauric, and capric acids can be useful to develop permeability of the incorporated compounds, including hydrophilic compounds such as insulin.³⁵

The important applications of chitosan-based entities and their applications are summarized in Table 1.2.

Table 1.2 Applications of chitosan-based entities.

S. n o.	Biomaterial	Application	Ref.
1	Cisplatin-loaded chitosan	Drug delivery in cancer therapy	36
2	Hydrophilic aspirin (ASA) drug and probucol (PRO)	Drug delivery	37
3	Doxorubicin-loaded chitosan NPs	Drug delivery	38
4	Black pomegranate peel extract loaded with chitosan-coated magnetic nanoparticles	Treatment of breast cancer cells	39
5	IFN-CT NPs	Administration of interferon-alpha (IFN α)	40
6	Chitosan–silica (CS–SiO ₂) hybrid microspheres	Vancomycin hydrochloride drug release	41
7	Chitosan-grafted-(cetyl alcohol–maleic anhydride–pyrazinamide) (CS- <i>g</i> -(CA–MA–PZA)	Rifampicin drug delivery	42
8	Chitosan–alginate polyelectrolyte complex (CS–ALG PEC) nanoparticles of	Amoxicillin drug delivery	43

	amoxicillin		
9	Chitosan (CS) polymer coated with reduced graphene oxide (rGO) and implanted with Fe_3O_4	Doxorubicin drug delivery	44
10	Chitosan-based hydrogels cross-linked with benzaldehyde	Release of caffeine, ascorbic acid, and 5-fluorouracil	45
11	Biotin-modified galactosylated chitosan nanoparticles (Bio-GC)	Drug release of 5-fluorouracil	46
12	PNIPA/chitosan core/shell nanoparticles	Controlled release of doxycycline hyclate	47
13	Poly(lactic acid) (PLA)/chitosan (CS) nanoparticles	Anti-HIV drug delivery	48
14	Chitosan (CS) nanoparticles incorporated with tripolyphosphate	Drug delivery	49
15	Chitosan-coated mesoporous silica nanospheres	Ibuprofen drug release	50
16	Chitosan nanoparticles stabilized with PEG	Drug delivery systems in cancer therapy	51
17	Chitosan–aniline oligomers and agarose-based electro-active hydrogels	Dexamethasone (DX) drug release	52
18	Chitosan hydrogels reinforced by silver nanoparticles	Wound healing applications	53
19	Savlon-loaded chitosan and PVA blends	Antibacterial activity	54
20	Chitosan (CS)/gelatin (Gel)/polyvinyl alcohol (PVA) hydrogels	Wound dressing	55
21	Chitosan, poly(<i>N</i> -vinylpyrrolidone) (PVP), and titanium dioxide (TiO_2) blends	Wound dressing	56

22	Chitosan, chitosan/gelatin, chitosan/ β -TCP and chitosan/gelatin/ β -TCP three-dimensional scaffolds	Antibacterial applications	57
23	Bio glass–natural biopolymer-based composite scaffold	Bone regeneration	58
24	Chitosan/58S-bioactive glass (58S-BG) containing poly(lactic-glycolic) acid (PLGA) nanoparticles	Bone tissue engineering	59
25	Chitosan/PPy–Alg scaffolds	Tissue regeneration	60
26	Thiolated chitosan-based chitosan–4-thioethylamine conjugate	Bone repair	61
27	Anisotropic chitosan/hydroxyapatite-based scaffolds	Bone repair	62
28	Carboxymethyl chitosan (CMCTS)– α -cyclodextrin conjugates	Bone tissue engineering	63
29	Chitosan/alginate/hydroxyapatite/nano-crystalline cellulose	Bone tissue culture	64
30	Nano-hydroxyapatite/collagen and poly(l-lactide)-based microsphere-scaffold	Delivery system for bone regeneration	65
31	Hydrogels from chitosan derivative (EGAMA-CS)/polyethylene glycol dimethacrylate (PEGDA)/N,N-dimethylacrylamide (DMMA)	Bone regeneration	66
32	CS/HA nano-composites	Bone tissue engineering	67
33	Chitosan Ag NPs scaffolds	Bone tissue engineering	68
34	Zirconium oxide (ZrO_2) doped nanocomposites with chitosan, organically modified montmorillonite (OMMT), and nano-hydroxyapatite	Bone tissue engineering	69
35	Chitosan/polyvinyl alcohol/graphene oxide/hydroxyapatite/gold films	Orthopedic applications	70

36	Chitosan (CHT) and hyaluronic acid (HA) scaffolds	Bone tissue engineering	71
37	Chitosan- <i>graft</i> -poly(l-lactide) (CS- <i>g</i> -PLLA) copolymers	Bone tissue engineering	72
38	Chitosan/agarose/hydroxyapatite nanocomposite scaffold	Bone regeneration	73
39	Chitosan-silk sericin 24/hydroxyapatite nanocomposites	Bone tissue engineering	74
40	Hydroxyapatite/graphene oxide/chitosan composite hydrogel	Bone tissue engineering	75
41	Polycaprolactone (PCL)/chitosan (CH) blend scaffolds	Nerve tissue engineering	76
42	Hydrogels of chitosan and silk fibroin	Tissue engineering scaffolds	77
43	Chitosan/poly(vinyl alcohol)(PVA)-based scaffolds	Tissue engineering	78
44	Polyvinyl alcohol (PVA)/chitosan nanofibrous scaffolds	Tissue engineering	79
45	Poly (phenylene sulfide) (PPS) and reduced graphene oxide (rGO)	Wound dressing	80
46	Chitosan- <i>graft</i> -poly(AA- <i>co</i> -AAm)/HAp nanocomposite scaffolds	Celecoxib drug release	81
47	Agarose/chitosan/graphene oxide (ACGO) composite scaffolds	Tissue engineering	82

1.5 Hyaluronic Acid

Hyaluronic acid (hyaluronan) occurs naturally in the eyes, skin, and bone joints. Chemically, it is glycosaminoglycan consisting of alternating *N*-acetyl glucosamine and α -glucuronic acid units linked by $\beta(1-4)$ and $\beta(1-3)$ linkages (Figure 1.3). It possesses molecular weights ranging from 6500 to 10 900 kDa.

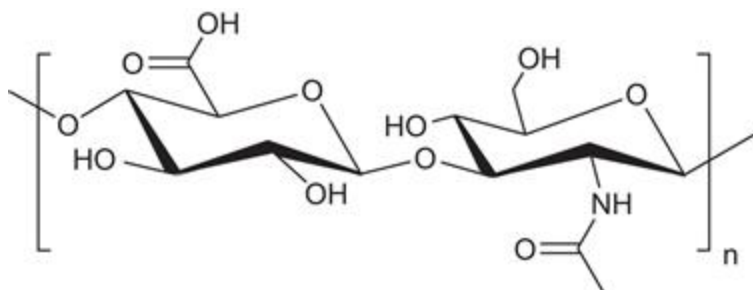


Figure 1.3 Chemical structure of HA.

The carboxylic and hydroxyl groups on the HA provide a platform for possible chemical modifications to articulate the mechanical and degradation properties of these materials. It is a major component of soft connective tissue ECM and helps in trapping water inside tissue cells, and keeps eyes moist. It also serves as a lubricant in joints. It also regulates the permeability of the blood vessel wall and promotes wound healing and moisturizing.

Hyaluronic acid (HA) has been widely used for the development of hydrogels due to its bio functionality and presence of modification sites. The modified HA macromers can form hydrogels *via* crosslinking through click chemistry or supramolecular interactions, and the developed HA hydrogels can be explored for the culture of cells and delivery of therapeutics.⁸³

HA, a biodegradable and non-toxic member of the glycosaminoglycan family serves as the important component of extracellular matrix and synovial fluid and serves in regulating cell adhesion, differentiation, and cell proliferation. Its bi-functionality allows structural modifications, making it an attractive material for medical products mainly hydrogels, cell therapy, and three-dimensional (3-D) cell cultures. Cross-linked structures formed *via* the covalent or non-covalent interactions help to improve stability, making it a suitable candidate for contact lenses, artificial corneas, and wound dressing.⁸⁴

Over just a few decades, HA has become a popular biomaterial in research and clinical applications. Cells can modify HA by regulating synthesis and degradation by the arsenal enzymes.⁸⁵

M. Gadomska *et al.* developed new materials from hyaluronic acid and albumin obtained from chicken eggs. The addition of egg albumin to hyaluronic acid decreases the mechanical properties of the developed materials. The developed materials are useful as biopolymers with good biocompatibility and biodegradability, and thus these materials can serve as adhesives.⁸⁶

Thermo-sensitive hydrogels developed by J.-P. Chen *et al.* were successfully used for intravesical cisplatin delivery into the bladder. Poly(*N*-isopropyl acrylamide) (PNIPAM) grafted onto HA and the addition of HA and gelatin produces a microstructure different from the parent PNIPAM hydrogel. *In vivo* evaluation of cisplatin levels in the bladder was studied and the tissues were observed to induce intravesical instillation in rats.⁸⁷

Hydrogels act as soft scaffolds to help tissues regenerate and heal. They are, therefore, widely used in tissue engineering and regenerative medicine. Supramolecular hydrogels based on non-covalent interactions have emerged as popular soft materials in the development of materials for biomedical use. The reversible and dynamic nature of these materials makes them injectable, responsive to stimuli, and applicable in 3D printing. The properties and bio-response of these hydrogels are different from those of chemically crosslinked hydrogels. Among various materials, HA constitutes a promising material in this field due to its functionality, hydrophilicity, and ease of chemical modification.⁸⁸

Hydrogels based on oxidized hyaluronic acid cross-linked with adipic acid dihydrazide can act as a suitable material in bioinks for 3D bioprinting. The gels can be mechanically stable and have a good printing ability.⁸⁹

Niosome non-viral vectors and hyaluronic acid (HA) hydrogel scaffolds were developed by Ilia V-B. *et al.* Various formulations of niosomes and different amounts of cationic lipid, helper lipid, and non-ionic tensioactives were developed as biocompatible materials. The results reveal that nioplex-loaded HA hydrogels show no particle aggregation, allowing the cell to spread with high cellular viability, and making them useful in *in vivo* local and non-viral gene delivery applications.⁹⁰

Hyaluronic acid (HA)-collagen sponge materials (HACSMs) consisting of several ratios of bird feet (BF) and pigskin (PS) collagen can be

fabricated by using freezing, lyophilizing, and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide crosslinking. B4P1HA (BF : PS : HA = 4 : 1 : 0.2) materials exhibit the highest value of tensile strength. There is no significant difference between B4P1HA and B5P0HA. The results support the hypothesis that the B4P1HA and B5P0HA materials can be tuned for the highest bio-stability and good mechanical strength. These materials are useful for artificial skin or drug delivery systems.⁹¹

HA hydrogels can also be obtained by cross-linking HA with divinyl sulfone (DVS) without using organic solvents. Studies show that sterilization and extrusion do not significantly alter the viscoelastic properties of the materials. These gels exhibit good stability against enzymatic degradation, and the stability increases with increasing HA concentration and lowering the HA/DVS weight ratio.⁹²

Biomimetic polymeric injectable hydrogels support nucleus pulposus (NP) regeneration and can be developed by using HA conjugated fibrinogen (FBG). The nucleotomized organ culture model and *in vitro* studies proved that the hydrogels of FBG–HA conjugates with 235 KDa HA at an FBG/HA w/w ratio of 17 : 1 exhibit good gel stability along with mechanical properties. The gene-expression levels of NP markers can be maintained *in vitro*. Specific hydrogels of FBG–HA based materials can be useful in injectable forms for minimally invasive and biological NP regeneration.⁹³

The layer-by-layer (LBL) self-assembly technique is a highly effective method for the immobilization of the main components of the extracellular matrix. Collagen and hyaluronic acid can be covalently immobilized on titanium-based implants to constitute a polyelectrolyte multilayer (PEM) film by electrostatic interaction. Results demonstrate that the covalent crosslinking enhances the chemical stability of the PEM film to a significant level. The increased stability and biological properties of the Col/HA PEM covalently immobilized are beneficial for the osseointegration of the implants.⁹⁴

Artificial tissue engineering scaffolds support the regrowth of severed axons following nerve injury. Biomaterials composed of alginate and hyaluronic acid are suitable for covalent modification and biocompatibility for living Schwann cells and are feasible for the construction of three-dimensional (3D) scaffolds. The double crosslinked composite hydrogels

allow Schwann cell survival and growth. The combination of hyaluronic acid with alginate can facilitate the fabrication process, and the 3D scaffolds can be fabricated from the composite hydrogels.⁹⁵

Hyaluronic acid and its derivatives are prominently used for the development of hydrogels. Hyaluronic acid hydrogels possess many advantages including promotion to cell adhesion and proliferation, excellent wound healing, *etc.* These materials also demonstrate sufficient biological activity to stimulate an environment for cell survival.⁹⁶

The cross-linking of high molecular weight hyaluronic acid (HMW-HA) and low molecular weight hyaluronic acid (LMW-HA) can be carried out with 1,4-butanediol diglycidyl ether (BDDE). The materials developed show good enzymatic stability, rheological characteristics and mechanical properties, and lower cytotoxicity, indicating their potential applications in regenerative medicine and tissue engineering.⁹⁷

HA is a primary component of the brain extracellular matrix that regulates cell behavior within the central nervous system (CNS) through cellular receptors. It is involved in several behaviors such as proliferation, migration, differentiation, inflammation, and homeostasis of the CNS. The biocompatibility of HA and its derivatives has served in augmenting the inherent regenerative processes within the CNS. Neural stem cells/progenitors are usually injected into CNS lesions for promoting tissue healing and functional regeneration. Dynamic cross-links, self-healing abilities, and shear-thinning make HA-based materials useful as ideal invasive injectability.⁹⁸

Injectable hydrogels based on HA can be filled in the defect area. They have good permeability and therefore constitute promising biomaterials for the delivery of various bioactive agents including proteins, growth factors, drugs, and living cells. Injectable hydrogels can also be used in invasive surgical procedures. Several cross-linking methods for the creation of various hydrogels based on HA were reviewed by S. Khunmanee *et al.*⁹⁹

Sustained and effective DNA delivery enhances the usefulness of gene therapy in therapeutic angiogenesis and tissue engineering. Porous hydrogel scaffolds can be encapsulated and used for the delivery of nucleotides in the form of nanoparticles to the target sites. Hydrogels of micro-porous (μ -pore)hyaluronic acid assist an effective cell seeding. The *in vitro* post-

scaffold fabrication can help for a long transgene expression of incorporated mMSCs term and a sustained transfection.

Cell viability can be observed for a high over time including at higher concentrations of DNA polyplexes. Hence, the μ -pore hyaluronic acid hydrogels can be used to design scaffold-mediated gene delivery in local gene therapy.¹⁰⁰

HA can induce small and significant changes in cellular viability. Hyaluronic acid may exert different effects in oral squamous cell carcinomas.¹⁰¹

Wound contact with seawater that has a high salt proportion and pathogenic bacteria may result in serious infections and difficulties in wound repair. Hyaluronic acid (HA) and quaternized chitosan (QCS) based composite hydrogels can promote wound healing in seawater-immersed wounds together with preventing bacterial infection. The addition of quaternized chitosan in the composite hydrogels of HA results in good swelling properties and mechanical abilities and enhanced antibacterial potential of the developed scaffolds. Antibacterial hydrogels decrease pro-inflammatory factors and enhance anti-inflammatory factors (TGF- β 1) in the wound.¹⁰²

pH-Sensitive bifunctional injectable composite hydrogel scaffolds for the delivery of growth factors can be used to improve the biofunctionability of hydrogel scaffolds. Diels–Alder click chemistry is useful for the design and development of HA hydrogels involving two types of crosslinking points. In comparison with pure hydrogels, composite hydrogels can lead to better results.¹⁰³

Many hydrogels comprised of gelatin, alginate, hyaluronic acid, *etc.* are available as bioinks in 3D printing in tissue engineering applications. Hydrogels of hydroxyethyl acrylate (HEA) and gelatin-methacryloyl with hyaluronic acid (HA) act as promising materials for the development of bio-printing gels. The 3D-printing of HA-*g*-pHEA–gelatin hydrogels has been reported to be successful, and the bone cells in bio-inks were viable.¹⁰⁴

The click-crosslinked hyaluronic acid (Cx-HA) hydrogel (Cx-HA-CM) consisting of chemically immobilized cytomodulin-2 (CM) is reported to be useful on human periodontal ligament stem cells (hPLSCs). The in situ formation of Cx-HA-CM hydrogels and hPLSCs has good potential in

cartilage tissue engineering. The CM remaining inside the Cx-HA may induce synergistic chondrogenic differentiation of hPLSCs useful in cartilage tissue engineering.¹⁰⁵

Hyaluronic acid and collagen are the prime constituents of the extracellular matrix in various tissues. The hydrogels composed of collagen and HA have inherent advantages due to their ability to mimic the structure and functioning of the native ECM. Recently, the methods of fabrication, properties, and applications of hydrogels were documented by Q. Xu *et al.*¹⁰⁶

Post-myocardial infarction (MI) initiates the adverse left ventricular (LV) remodeling process which may result in dilation, loss of contractile function, and symptomatic heart failure. Functionalized and cross-linked hyaluronic acid (HA) may exhibit controlled and tunable mechanics and can be assessed for the temporal dependency of mechanical stabilization in LV remodeling.¹⁰⁷

1.6 Dextran

Dextran is a polysaccharide composed of α -1,6 linked α -glucopyranose including some degree of branching *via* α -1,3 linkage. It is a carbohydrate-based natural polymer found in microorganisms, plants, and animals. It can be dissolved in water and DMSO and has a lower viscosity than starch; hence it serves as a good material for chemical modifications including biomaterials. On account of its non-toxicity, solubility in water, biodegradability, biocompatibility, immunomodulation, and ease of chemical modification, it is used for the development of biomaterials. The presence of reactive hydroxyl functional groups in dextran provides an avenue for the development of scaffolds in the form of tubular, spherical, and 3D network structures.

Dextran-based scaffolds, being naturally biodegradable, can act as bioactive carriers for the controlled release of various biomolecules in wound healing and tissue engineering.¹⁰⁸

Polymeric conjugates of the carboxymethyl dextran (CMD) backbone with ovalbumin (OVA) can act as a foreign antigen and are useful in cancer immunotherapy. The *in vitro* cellular uptake studies reveal that the

conjugate is taken up by TC-1 cervical cancer cells. The CMD-OVA was investigated to significantly induce higher antigen presentation at the tumor site.¹⁰⁹

A photo-degrading ability of dextran-glycidyl methacrylate hydrogels was documented by C.-W. Lo *et al.*¹¹⁰

PEG/dextran double layers can influence the release of iron ions and the colloidal stability of iron oxide nanoparticles. A double-layered polymeric corona composed of a dextran interior surrounded by PEG as an exterior shields the core polymer-coated iron oxide nanoparticles. The PEGylated samples reveal the reduction of bio-distribution in the liver and spleen and show no influence on NP blood retention.¹¹¹

Dextran (Dex) nanohydrogels (NGs) grafted with polyacrylic acid (PAA) involving covalently crosslinked structures containing redox-sensitive disulfide crosslinking junctions (Dex-SS-PAA) were developed by H. Wang *et al.* Doxorubicin was conjugated with Dex-SS-PAA by an acid-labile hydrazone bond (Dex-SS-PAA-DOX) and was useful in the *in vitro* pH responsive and redox controlled release of the drug. The developed system can inhibit MDA-MB-231 tumor growth.¹¹²

Thermo-sensitive hydrogel networks of dextran and *N*-isopropyl acrylamide can be achieved using gamma radiation in the presence of an initiator/cross-linker agent. The membranes obtained can be loaded with OndansetronTM and used as a drug delivery system for antiemetic drugs.¹¹³

Praziquantel is an anthelmintic drug used for the treatment of schistosomiasis. Despite the high permeability, it has lower solubility in an aqueous medium, limiting its bioavailability. Dextran hydrogels comprised of polymer blends with 70 and 148 kDa dextrans in various proportions of praziquantel can be used for improving the solubility of poorly water-soluble drugs.¹¹⁴

Hybrid core shell nano-carriers of hydroxyapatite-oxidized dextran methacrylate can serve as for dual drug delivery applications. The combinatorial drug delivery of doxorubicin (DOX) and methotrexate (MTX) useful in the treatment of bone cancer can be useful in chemotherapy. *In vitro* drug release studies supported the release of MTX followed by DOX within 10 days.¹¹⁵

2-Acrylamido-2-methyl-1-propane sulfonic acid (AMPS) grafted biodegradable dextran involving poly-AMPS chains on the backbone of dextran can be developed by free radical polymerization in the presence of ceric ammonium nitrate initiator.¹¹⁶

Dextran can act as an effective drug delivery carrier and inhibit cancer cells effectively together with a reduction in toxicity and side effects of the drugs in biological systems. It also helps to release the antitumor drugs slowly.¹¹⁷

Supermacroporous dextran gels (SDGs) obtained by the dextran macromonomer *via* radical polymerization have a fast swelling ability and can be useful for tissue engineering and cell culture.¹¹⁸

Semi-interpenetrating networks of hyaluronic acid with hydroxyethyl-methacrylate-derivatized dextran (dex-HEMA) can result in the formation of 3D hydrogels, which can be used for bio-printed constructs in tissue engineering.¹¹⁹

Biodegradable and injectable hydrogels consisting of cross-linking of aldehyde-functionalized dextran, hydrazide-functionalized β -cyclodextrin and hydrazide-functionalized dextran can be used in hydrophobic drug delivery. The low cytotoxicity and injectable nature of these materials make them attractive drug delivery systems.¹²⁰

Hydrogels obtained by crosslinking dextran with amino acid diamines can show pH-responsive behavior and higher swelling at more basic pH, acting similarly to the anionic hydrogels.¹²¹

The hydrogels of dextran incorporated with praziquantel as a model drug can be useful for the controlled release of drugs.¹²²

Paclitaxel (PTX) can be released in a controlled manner by incorporating PTX into acetylated dextran (Ac-DEX) nanoparticles. This approach constitutes a convenient and effective strategy for spinal cord injury therapy.¹²³

1.7 Alginates

Alginates are unbranched and linear polyanionic polysaccharides consisting of 1,4'-linked β -D-mannuronic and α -L-guluronic acid. These are natural polysaccharides consisting of guluronate and mannuronate units. They can

be extracted from seaweed and are usually obtained from brown seaweeds such as *Lessonia* sp., *Macrocystis* sp., and *Laminaria* sp. Alginates can also be obtained using a fermentation process. Alginates can be articulated in the form of injectable solutions, sponges, fibers, and hydrogels for drug delivery and tissue engineering applications.

Alginate is an edible heteropolysaccharide. Due to its gel-forming ability, alginate is used in the textile, food, and paper industries and to a lesser extent in biomedical applications including wound healing and tissue regeneration. Alginate can be made softer by converting it into composites. The addition of natural polymer, synthetic polymer, or adhesive peptide to alginate results in better mechanical properties and enhances the healing ability of the material. Biomedical applications of alginates have been recently discussed by R. A. Raus *et al.*¹²⁴

Alginate hydrogels can be used as attractive materials in drug delivery, wound healing, and tissue engineering applications.¹²⁵

Alginates can be prepared from various methods and possess attractive properties as biomaterials useful in wound healing, tissue repair, and drug delivery.^{126,127}

The properties of alginate di-aldehyde (ADA) gels can be controlled and made into suitable materials for long-term cell culture investigations in cartilage, bone, and blood vessel engineering for bio-fabrication processes.¹²⁸

The process of wound healing involves a complex governed by various factors such as bacterial contamination, depth of the wound, and systemic factors. G. C. Türkoğlu *et al.* developed textile–hydrogel hybrids by using natural-based sodium alginate and chitosan polymers.¹²⁹

The application of carbon-based nano-materials and aerogels is increasing in the tissue engineering field. Highly porous collagen–alginate (graphene oxide) aerogel-based scaffolds can be developed for a supercritical drying process. The promotion of cell attachment and proliferation by a collagen–alginate scaffold is supported based on SEM analysis.¹³⁰

The addition of rubidium into a calcium alginate hydrogel followed by dressing with freeze-drying enhances the roughness and *in vitro* degradation rate. Such materials have good bactericidal action against *S. aureus* and *P.*

aeruginosa. These materials are non-toxic and conducive with human umbilical vein endothelial cells.¹³¹

Hydrogels obtained by the combination of hyaluronic acid and alginate possess enhanced efficiency of the biocompatible matrices with tailored properties.¹³²

The sodium alginate/PVA hydrogel-based scaffolds incorporated with bFGF encapsulated microspheres can be used for accelerating the process of wound healing. Increasing the concentration of sodium alginate in the hydrogel results in a more porous structure and higher swelling ability. The developed system is non-toxic according to the MTT assay; therefore, the developed materials are useful in the treatment of wound healing.¹³³

A. Mohandas *et al.* developed an alginate hydrogel/zinc oxide nanoparticle composite bandage using a freeze-drying technique. The composite has a porosity of 60–70%. The swelling ratios of the bandages decrease with an increasing amount of nZnO in the scaffold. The composite bandages showed excellent antimicrobial activity against *S. aureus*, *E. coli*, *C. albicans*, and methicillin-resistant *S. aureus* (MRSA). The composites exhibit no toxic effects toward human dermal fibroblast cells at lower concentrations of nZnO.¹³⁴

1.8 Pullulan

Pullulan is a non-toxic, water-soluble, and biocompatible exopolysaccharide. It is easily biodegradable and contains active hydroxyl groups. Chemically, it is a natural polysaccharide having maltotriose units linked *via* α -(1 $\rightarrow$ 4) and α -(1 $\rightarrow$ 6) linkages. Hydrophobic groups can be easily connected by chemical modification *via* esterification resulting in amphiphilic graft copolymers. It is mainly produced by the yeast-like fungus *Aureobasidium pullulans*.

Infected tissues can be treated with pullulan–drug conjugates. They show high bioactivity for the controlled release of cytotoxic entities. Pullulan conjugates can be useful in the targeted drug and gene delivery in the treatment of various diseases of the liver, brain, spleen, and lungs.¹³⁵

Cholesteric hydrophobically modified pullulan, CH-modified carboxylated pullulan, and CH-modified animated pullulan nanoparticles

can be synthesized by dialysis. The surface charges and size of NPs play an important role in drug release.¹³⁶

Polysaccharides are widely used biomaterials for the development of drug and gene delivery systems on account of their structural flexibility, biodegradability, and biocompatibility. They are also used in the transmucosal delivery of proteins. Pullulan can be used for the production of nanoparticles in protein delivery.¹³⁷

Cholesteryl-modified aminated pullulan nanoparticles can be evaluated for drug release. Cytotoxicity findings show the best inhibition of Lewis lung cancer cells.¹³⁸

Polymeric colloidal nanocarriers obtained from hydrophobically grafted carbohydrates can be used to increase the percutaneous penetration of hydrophilic actives. Hydrophobically grafted pullulan (BMOPUL) derivatives with 2-(butoxymethyl)oxirane afford monodispersed BMO-PUL nanocarriers. The systems are stable and can work at physiologically relevant pH. *In vitro* investigations of these materials show a lack of toxicity, highlighting the potential ability of these materials as nanocarriers for percutaneous delivery applications.¹³⁹

Pullulan can form micro-particles, films, nanoparticles, hydrogels, and micelles. Pullulan can be produced from starch by the fungus *Aureobasidium pullulans*. The structure of pullulan can be modified for the release of drugs.¹⁴⁰

Cholesterol-modified pullulan self-aggregated nanoparticles (CHSPNs) can be developed as drug nanocarriers. A single-dose toxicity test performed in mice shows that CHSPNs are well tolerated at a dose of 200 mg kg⁻¹, indicating good stability and safety. This indicates the promising nature of cholesterol-modified pullulan self-aggregated nanoparticles as a stable, safe, and potential drug nano-carrier.¹⁴¹

A pullulan-based pH-sensitive intracellular nanoparticle carrier system was developed by Y. Wang *et al.* Pullulan derivatives were synthesized by conjugating urocanic acid and cholesterol succinate to the pullulan. These systems show amphiphilic and pH-sensitive properties in the range of 6.5.¹⁴²

Pullulan–polymethacrylate associations in the form of films can be developed as a target-specific material for enteric drug release. The

polysaccharide concentration affects the vapor permeability and swelling ability. The developed films were thermally stable possessing good mechanical properties. Drug release is dependent on the synergism between pH and enzyme dependence.¹⁴³

1.9 Chondroitin Sulfate

Chondroitin sulfate, an acidic glycosaminoglycan, is an important constituent of blood vessel walls, connective tissues, cartilage, skin, and tendons. Chemically, these branched polysaccharides consist of alternating units of β -glucuronic acid and *N*-acetylgalactosamine units. The polysaccharide affords cushioning properties to the cartilage, a water retention ability, and hence acts as a lubricant at the sites of articulating surfaces.

Chondroitin sulfate has become a popular material in drug delivery and tissue engineering applications due to its biodegradable and non-toxic nature.^{144,145}

The combination of extracellular matrix molecules derived hydrogels and ECM-derived ligands exhibits synergistic effects useful in the delivery of intracellular drugs.¹⁴⁶

Chondroitin sulfate–methotrexate nano-gels can be developed by bio-conjugating methotrexate (MTX) with chondroitin sulfate. The nanogels enhance the solubility and improve the delivery efficacy of MTX due to binding to CD44. The bio-conjugate enhances the solubility of sparingly soluble drugs and is also useful in targeted delivery to cancer cells and tumor tissues.¹⁴⁷

T. Ramasamy reported the formulation and evaluation of chondroitin sulfate tablets of aceclofenac and their application against colon-targeted drug delivery, indicating the potential of the developed system as an aceclofenac carrier by regulating drug release in the small intestine and stomach.¹⁴⁸

Chondroitin sulfate-loaded sodium alginate/chitosan composite nanoparticles can be used as drug carriers that can pass through the stomach and release drugs into the intestinal tract.¹⁴⁹

The Golgi apparatus plays an important role in signaling multiple pathways. A prodrug nanoparticle system targeting the Golgi apparatus can be developed by synthesizing chondroitin sulfate conjugated with retinoic acid.¹⁵⁰

Recently, a ternary hydrogel of gellan gum (GG), silk fibroin, and chondroitin sulfate developed by a physical and chemical crosslinking method was documented as a biomaterial for cartilage tissue engineering. High proportions of GG result in enhanced mechanical properties of the hydrogel. The combination of these biopolymers synergistically promotes articular cartilage defect repair and thus constitutes a promising material for application as a biomaterial for cartilage tissue engineering.¹⁵¹

A catecholamine in marine mussels has good adhesiveness and cohesiveness. Catechol conjugated chondroitin sulfate can serve as an avenue for mesenchymal stem cell culture. Immortalized human mesenchymal stem cells revealed the high cytocompatibility of the biopolymer, making the material a suitable bioink for bio-printing applications.¹⁵²

J. Xi *et al.* developed a chondroitin sulfate functionalized mesostructured silica nanoparticle (NMChS-MSNs)-based pH-responsive drug carrier system. The NMChS-MSNs were well dispersed in aqueous solution, favoring the drug carrier of Doxorubicin hydrochloride as a controlled drug delivery system.¹⁵³

Chondroitin sulfate molecules can be modified with the *N*-hydroxy succinimide (NHS) group. The *ex vivo* cornea model supports the down-regulation of the pro-inflammatory gene expression of keratocytes, indicating the potential ability of the cross-linked material as an effective, biocompatible, and safe means of corneal reinforcement.¹⁵⁴

Curcumin can be encapsulated into polymeric nanoparticles (NPs) and conjugated with chondroitin sulfate to their surfaces. The developed system results in a negatively-charged surface and monodisperse size distribution and reveals excellent biocompatibility and results in a higher cell internalization efficiency.¹⁵⁵

1.10 Conclusion

Most of the natural polymers are present as extra cellular matrix components of organisms and are involved in inter- and intra-cellular cell signaling thereby contributing to the cell growth. These polymers have a unique biological potential due to their ease of availability, natural abundance, possibilities for structural modifications, biodegradability, and biocompatibility. Most of them can be classified as biomaterials with good bioactivity and mechanical strength and can be processed in the form of gels, films, composites, and sheets. Besides the presence of available sites for structural modification, semi-synthetic polymers also offer several advantages. In summary, naturally occurring polysaccharides are biomaterials of great importance in biomedical applications useful in drug delivery, wound healing, as well as bone tissue engineering applications and will continue to be materials of high significance in the future.

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Chapter 2

Recent Approaches in Alginate-based Carriers for Delivery of Therapeutics and Biomedicine

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2.1 Introduction

Alginates are a group of polysaccharides that are widely found in nature. Approximately 40% by dry weight of alginic acid is part of the structure in

brown marine algae (Phaeophyceae). Also, alginates can be found as capsular polysaccharides in soil bacteria.¹ As they are located both in the cell wall and in the intercellular matrix of brown algae, the alginates give it mechanical properties such as resistance and elasticity, thus helping the algae to survive in the sea. In general, alginates are used in the form of sodium due to their ability to solubilize in cold water, and because of their ease of binding with the salts that are present in ocean water (like Ca^{2+} , Na^+ , Mg^{2+} , Sr^{2+} , and Ba^{2+} ions).^{2,3} Around the world there are different kinds of brown seaweed, and from these the alginates are extracted; among the most used for their commercial extraction are *Sargassum* spp., *Fucales* spp., *Laminaria japonica*, *Laminaria digitata* and *Ascophyllum nodosum*. The quality of the alginates produced depends significantly on the kind of seaweed species used, as well as the climatic conditions during the growth stage, the harvest season, and the manufacturing process.^{4,5} Some authors have mentioned that alginates obtained by biological synthesis have chemical structures that are more homogeneous and better physical properties than those extracted from brown seaweed.⁶ Due to their nature and functionality, they are widely used in different applications (cosmetics, textiles, foods, biomedical, and pharmaceuticals). In this chapter, we present the main alginate extraction methods, their physicochemical and functional properties, as well as a review of its different applications in the delivery of therapeutic compounds, delivery drugs, and applications in the field of biomedicine over recent years (Figure 2.1)

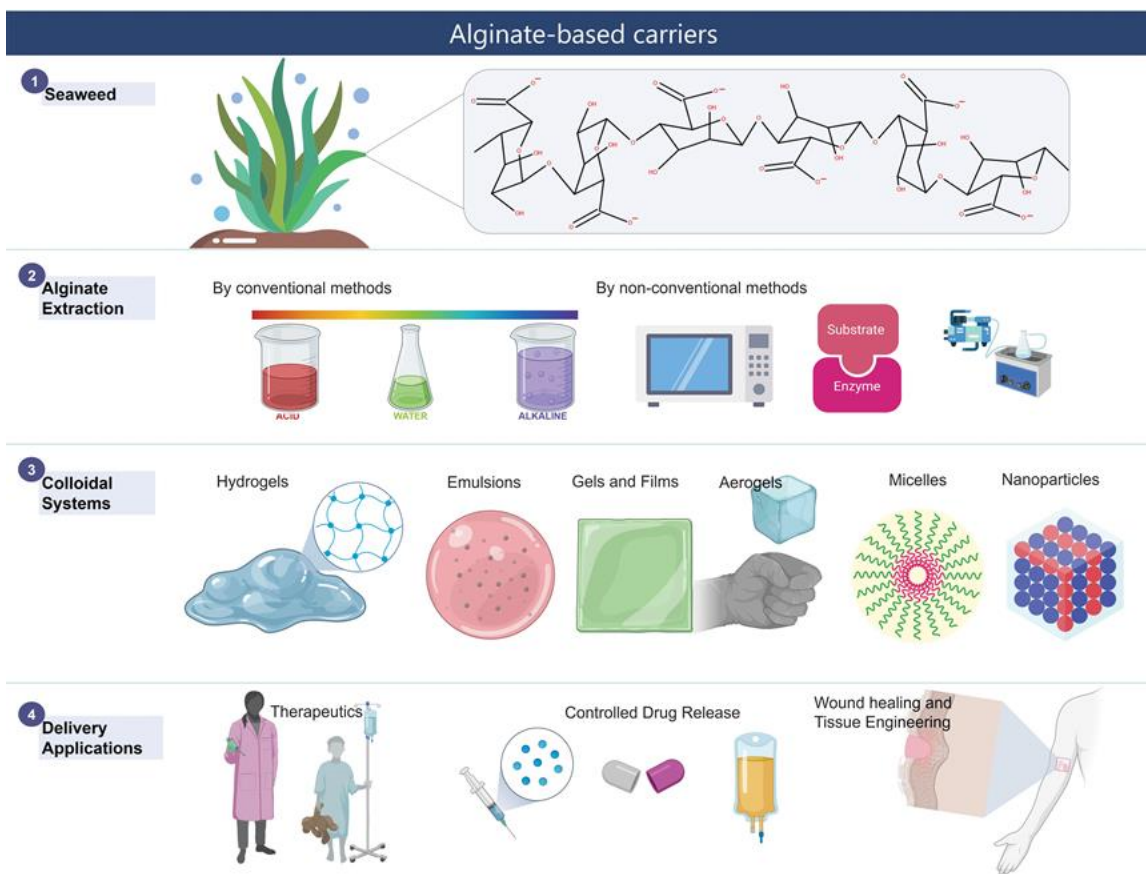


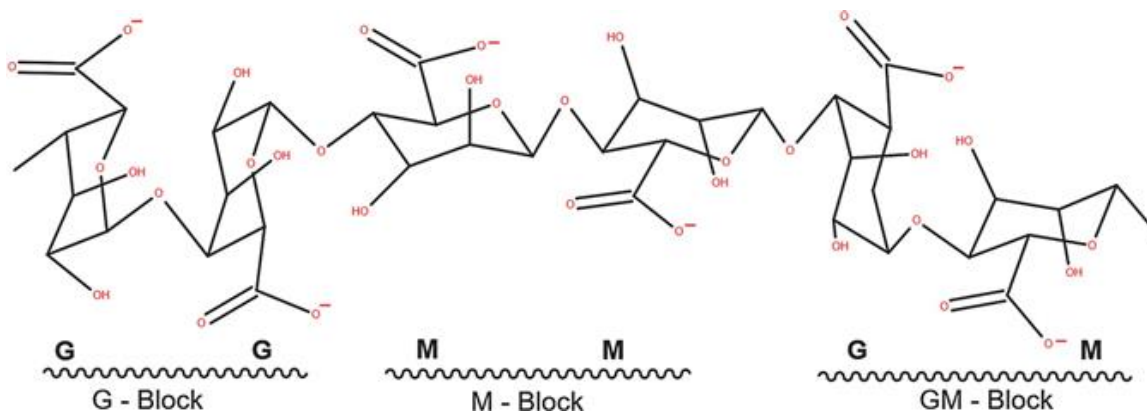
Figure 2.1 Recent applications of alginate-based carriers for therapeutics and drugs and in biomedicine.

2.2 Alginate

2.2.1 Alginate: Chemical Structure and Characterization

It has been extensively investigated that depending on the seaweed species, the age of population and the geographic location and season, the chemical structures of alginate differ. Alginate is considered an unbranched binary copolymer made-up of α -L-guluronic acid (G) with (1 $\rightarrow$ 4)-linked β -D-mannuronic acid (M) residues as monomers irregularly block-wise ordered along a chain establishing sequential block structures (M-, G-, and MG-) (Scheme 2.1)^{2,7} which could differ from the plant tissue and algal species, where the M/G ratio and block structure endow significant effects on the physicochemical and functional properties.^{8,9} The molecular weight ranges

generally between 500 and 1000 kDa. The presence of divalent ions, concentration, pH, ionic force, among other factors, affect alginate solubility.^{8,10}



Scheme 2.1 Chemical structure of alginate.

2.2.2 Extraction of Alginate from Brown Seaweed Algae

Alginates are extracted for obtaining an industrial product with controlled molecular weight and high yields.⁴ These polysaccharides are alginic acid salts (Ca^{2+} , Mg^{2+} , and Na^{1+}) found in the cell walls of brown algae.² The extraction of alginate from seaweed consists of transforming all the alginate salts to sodium alginate before its recovery in a water solution because calcium and magnesium salts are insoluble. Extraction methods can be conventional when solvents (water, acids, alkaline solutions) are used, and non-conventional when technologies such as enzyme- (EAE), microwave- (MAE), pressurized liquid (PLE), or ultrasound- (UAE) assisted extractions are considered.

Notwithstanding the type of extraction method, several steps must be considered.^{2,4} Among the pretreatment steps, seaweed is dried by heat, vacuum, or freeze-drying methods, and the cell walls exhibit high rigidity, so it is necessary to cause disruption (thermal, chemical, mechanical, enzymatic, ultrasound, microwave, *etc.*) to facilitate the extraction of the inner components. Then, the removal of lipids, pigments, and low molecular weight components from seaweed is carried out using solvents with different

polarities, ensuring the integrity of the alginate structure. The crude extract from seaweed is further extracted to obtain alginate.

2.2.2.1 *Alginate Extraction by Conventional Methods*

Conventional extraction (CE) of alginate usually consists of the use of water, acidic, or alkaline solutions at different temperatures during prolonged times. The main variables in the process involve the pH, temperature, particle size, solvent, and sample: solvent ratio.⁴ Aqueous extraction uses water at 80–100 °C, and the main disadvantage is related to the extraction of water-soluble compounds from the seaweed, affecting the number of purification steps. Some strategies used for improving the extraction yield involve the use of HCl (0.1–0.2 M) or H₂SO₄ solutions, because they induce cell wall hydrolysis, facilitating fucoidan and laminarin polysaccharide extraction.^{11,12} Besides, contributing to have alginic acid derived from alginate, which is insoluble in water and, together with solid seaweed residues, is removed, obtaining a purified fucoidan fraction.¹³ Sodium carbonate (Na₂CO₃) is utilized to treat the remaining solid residue, thus stimulating the formation of sodium alginate (SA) from alginic acid. SA is recovered by precipitation in ethanol and then submitted to a drying process to obtain a powder.^{5,14} Table 2.1 summarizes the extraction conditions of alginate from brown algae through CE methods.^{1,2,5,8,11,12,14–21}

Table 2.1 Conventional methods for the extraction of sodium alginate.

Algae (brown seaweed) (country or region)	Pretreatment conditions	Extraction conditions and purification	Drying process	Yield (% w/w)	Ref.
<i>Sargassum filipendula</i> (Brazil)	Formaldehyde (0.4% w/w) for 0.5 h. HCl 0.1 M for 2 h.	Na ₂ CO ₃ solution (2% w/w) at 60 °C. EtOH precipitation	—	15.1 ± 0.1 to 17.2 ± 0.3	Bertagnolli <i>et al.</i> ¹
<i>Sargassum filipendula</i> (Brazil)	Formaldehyde (0.4% w/w) for 30 min HCl 0.1 M for 2 h.	Na ₂ CO ₃ solution (2% w/w), 5 h at 60 °C×2. EtOH (1 : 1) v/v precipitation	—	24 ± 5.00	Costa <i>et al.</i> ¹⁵
<i>Sargassum</i> spp. (Brazil)	85% EtOH for 3 h×2 HCl (0.1 M, pH 2.0,) for 110 min at 45 °C.	Na ₂ CO ₃ solution (2% w/v) pH 10, 90 min at 75.7 °C. EtOH (2 vol.) precipitation	Convection, 45 °C for 12 h	11 ± 1.88 —	Yamashita <i>et al.</i> ¹⁴
<i>Cystoseira barbata</i> (Romanian)	70% EtOH v/v, 24 h. HCl (0.1 M) for 2 h at 60 °C.	Na ₂ CO ₃ solution (3% w/v) 2 h at 60 °C. EtOH (96%, -18 °C) (3v) precipitation	Convection, 50 °C	19 ± 1.5	Trica <i>et al.</i> ¹¹
<i>Carpophyllum flexuosum</i> , <i>Carpophyllum plumosum</i> , <i>Ecklonia radiata</i> , and <i>Undaria</i>	Milli-Q water at room temperature	Na ₂ CO ₃ solution (1.5% w/w) 1 h at 60 °C. EtOH precipitation	Freeze-drying	5.2–15.5	Zhang <i>et al.</i> ¹⁶
<i>Sargassum angustifolium</i> (Iran)	85% EtOH at 22 ± 2 °C. Distilled water (65 °C, 3 h). HCl (0.1 M, pH 2, 65 °C, 3 h). Alcalase (5% w/w, pH 8, 50 °C, 24 h). Cellulase (5% w/w, pH 4.5, 50 °C, 24 h).	Na ₂ CO ₃ solution (3% w/w, pH 11) 3 h at 65 °C. EtOH precipitation	Convection, 22 ± 2 °C	3.30–3.50	Borazjani <i>et al.</i> ⁵
<i>Colpomenia peregrine</i> (Iran)	85% EtOH 4 h at 22 ± 2 °C×2. Distilled water (65 °C, 3 h×3).	Na ₂ CO ₃ solution (3% w/w, pH 11) 3 h at 65 °C.	Vacuum at 60 °C.	3.80–6.60	Rostami <i>et al.</i> ¹⁷
<i>Padina pavonica</i> (Tunisia)	HCl (0.1 M, pH 2, 65 °C, 3 h×3). Alcalase (5% w/w, pH 8, 50 °C, 24 h). Cellulase (5% w/w, pH 4.5, 50 °C, 24 h). H ₂ SO ₄ (pH 1.5)	EtOH precipitation Na ₂ CO ₃ solution (4% w/w) 48 h at 65 °C. EtOH (1 : 4 v/v) precipitation	Freeze-drying	—	Faidi <i>et al.</i> ¹⁸
<i>Sargassum natans</i> , <i>Sargassum vulgare</i> , <i>Padina</i> <i>gymnospora</i> , and <i>Padina</i> <i>antillarum</i> (Ghana)	Formaldehyde (2% w/w) for 30 min HCl 0.2 M.	Na ₂ CO ₃ solution (2% w/w) 3 h at 99 °C. Isopropanol (80%)	Freeze-drying	16–29	Rhein- Knudsen <i>et al.</i> ²⁰ Rhein- Knudsen <i>et al.</i> ¹⁹
<i>Sargassum latifolium</i> (Egypt)	Citric acid solution (2% w/w) for 2 h at room temperature	Na ₂ CO ₃ solution (3% w/w), 1–3 h, 25–45 °C. EtOH (1 : 2 v/v) precipitation	Natural convection	18.89–40.43	Fawzy <i>et al.</i> ²¹
<i>Laminaria digitata</i> (Morocco)	Formaldehyde (2% w/w).	Na ₂ CO ₃ solution (2% w/w) 5 h at 25, 40 and 60 °C. EtOH precipitation (2 vol.)	—	35.28–51.80	Fertah <i>et al.</i> ²
<i>Nizimuddiniazanardini</i> (Iran)	HCl solution 0.2 M for 24 h. Formaldehyde (2% w/w) for 24 h.	Na ₂ CO ₃ solution (3% w/w) 2.5 h at 60 °C. EtOH precipitation	Freeze drying	24	Khajouei <i>et al.</i> ⁸
<i>Sargassum</i> seaweed (Caribbean region)	HCl 0.2 M for 3 h at 60 °C. Formaldehyde (2% w/w) for 24 h. Mohammed <i>et al.</i> ¹²	Na ₂ CO ₃ solution (5–10% w/v) 0.5–6 h between 25 and 75 °C. H ₂ SO ₄ 0.1–1 M, for 1 h between 25 and 60 °C.	Freeze drying EtOH precipitation	28.16 ± 0.97	

2.2.2.2 Alginate Extraction by Non-conventional Methods

Because CE sometimes consumes large amounts of solvents and energy, the use of non-conventional extraction techniques like MAE, UAE, enzyme assisted extraction (EAE), and subcritical water extraction (SWE), has overcome some limitations inherent to CE procedures, improving the

extraction yields, shortening the extraction times, reducing energy consumption, and lowering costs.⁴

MAE is considered one of the most efficient extraction processes, and it consists in removing solutes from a solid matrix into a solvent. The regulated and precise heat is due to the microwave radiation capacity to pass through the substrate and merge with it. Some advantages of this technique include high quality products, yield upgrade, product uniformity enhancement, energy-saving, and manufacturing costs and processing time reduction. During microwaving, dipole rotation and/or dissolved ions of the polar solvent heat the material directly by ionic conduction, but only polar compounds are affected. The rupture of the cell wall and the release of intracellular compounds into the extraction solvent are caused by the effective internal heating.²² The most relevant extraction parameters are microwave power, irradiation time, temperature, and pressure.⁴

As a result of its simplicity, decreased costs, accelerated extraction time, great yields, and shorter times, at an industrial scale UAE is the most useful technique.²³ It is based on the acoustic cavitation that causes a decrease in particle size, superior contact with the solvent and cell disruption produced by physical forces like acoustic streaming, micro-jets, shockwaves, and shear.^{24–26} The main parameters considered for UAE are solvent, solid/liquid ratio, particle size, and sonication time and amplitude.

EAE is a useful technique employed for bioactive compound extraction, and among its main advantages are the easy recuperation and less usage of solvent compared to CE, energy-saving, better yields, and quick extraction.²⁷ EAE for polysaccharides requires enzymes capable of full or partial degradation of cell walls or lead to the enzymatic hydrolysis of polysaccharides into smaller fragments.⁴

A recent method of extraction is PLE, which needs high pressures and temperatures for alginate extraction under the presence of oxygen and a light-free environment, requiring shorter times and fewer solvent amounts. Elevated temperature generally increases the solubility of samples leading to higher diffusion rates and the boiling point of the solvent is maintained below with the high pressure.²⁸ PLE could be classified as accelerated solvent extraction (ASE), hot water extraction (PHWE), pressurized-solvent extraction (PSE), pressurized fluid extraction (PFE), or subcritical water

extraction (SWE) according to the extraction conditions and the solvent.⁴Table 2.2 summarizes the conditions for non-conventional methods for alginate extraction.^{13,29–31}

Table 2.2 Non-conventional methods for the extraction of sodium alginate.^a

Algae (brown seaweed) (Country)	Pretreatment and extraction conditions	Purification	Drying Process	Yield (%w/w)	Ref.
<i>Sargassum binderi</i> and <i>Turbinariaornata</i> (Mauritius)	80% EtOH at room temperature. 2% NaOH UEA (150 W; 30 min) at 90 °C	1 N HCl at room temperature . Na ₂ CO ₃ solution (0.5% w/w). EtOH precipitation; 5% CaCl ₂	Freeze-drying	55	Yousouf <i>et al.</i> ¹³

<i>Ascophyllum nodosum</i>	80% EtOH (v/v) for 20 h at (21–23 °C).	Na ₂ CO ₃ solution (2% w/w), 5 h at 60 °C × 2.	Freeze-drying	70 ± 4	Okolie et al. ²⁹
	Blend at 70 °C for 5 h × 2.				
	0.01 M HCl	95% (v/v) ethanol; precipitation for 3 h at room temperature			
	UEA (20 kHz; 750 W; 40% amplitude for 35 min at 20 °C) × 3 each 20 min				
<i>Ascophyllum nodosum</i>	80% EtOH (v/v) for 20 h at (21–23 °C).	Na ₂ CO ₃ solution (3% w/w), 10 min at 100 °C × 3.	Freeze-drying	56 ± 1	Okolie et al. ²⁹
	Blend at 70 °C for 5 h × 2.				
	0.01 M HCl	95% (v/v) ethanol; precipitation for 3 h at room temperature			

	MEA (15 min at 90 °C) × 3 each 20 min				
<i>Ascophyllum nodosum</i>	80% EtOH (v/v) for 20 h at (21–23 °C).	Na ₂ CO ₃ solution (3% w/w), 3 h at 70 °C × 3.	Freeze-drying	90 ± 5 g	Okolie <i>et al.</i> ²⁹
	Blend at 70 °C for 5 h × 2.				
	EAE/CCE; 0.1 M sodium acetate buffer (pH 4.5) at 50 °C.	95% (v/v) ethanol; precipitation for 3 h at room temperature			
	Cellulase (pH 4.5, 50 °C) for 24 h				
<i>Sargassum Muticum</i> (Spain)	SWE; distilled water in a stainless stirred reactor using a liquid : solid ratio 30 : 1 w/w at 150 or 170 °C.	Precipitation by 1% CaCl ₂	—	—	Flórez-Fernández <i>et al.</i> ³⁰

<i>Saccharina japonica</i> (Republic of Korea)	PHWE; hot water extraction at 180–420 °C and pressure between 13 and 520 bar for 30–105 min	—	Freeze-drying	72.21 to 98.91	Saravana <i>et al.</i> ³¹
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^a Ultrasonic extraction assisted (UEA); microwave extraction assisted (MEA); enzyme assisted extraction (EAE); subcritical water extraction (SWE); pressurized hot water extraction (PHWE).

2.3 Alginate-based Colloidal Systems

2.3.1 Alginate in Different Colloidal Systems

In recent years, the development and research of materials with potential applications in traditional medicine and biomedicine, and the use of hydrocolloidal alginate-based systems have demonstrated very promising functional and biological properties. This biocompatible polysaccharide from ionic nature is employed for a great number of applications in engineering and biological areas, including the regeneration of articular cartilages, therapeutic delivery, drug delivery, and administration systems for wound healing, scaffold for cell growth, and tissue engineering.³² Most of these applications involve the use of colloidal systems, therefore a brief description of them is discussed below.

2.3.1.1 Hydrogels

Biopolymer hydrogels are characterized by their hydrophilic character and biocompatibility, and they can soak water up several times their weightiness on a dried basis, swell, and hold aqueous fluids in large amounts, be highly chemically stable, and may be degraded, disintegrated, or eventually dissolved. In terms of structure, hydrogels are considered as reversible or physical gels formed by environmental stimuli like salt concentration,

temperature, acidity (pH), or by interactions of a physicochemical nature like stereochemical complexation, electrostatic and hydrophobic interactions, or molecular self-assembly chemistry.³³

Hydrogels based on natural polymers owe their functionality to their capability for creating strong enough interactions between the chains and forming semi-permanent junctions within the molecular structure, creating a network that holds a large number of water molecules, and maintaining their biological compatibility, degradability, anchoring, scaffolding, stiffening, bio-absorbability, and affordable price.^{32,34} These attributes allow designing carrying and delivering systems with a specific target recognition or capability for self-disruption by environmental conditions.

Alginate hydrogel formation is usually carried out by the ionotropic mechanism, where SA coordinates with divalent or polyvalent metal cations like Mg^{2+} , Ca^{2+} , or Ba^{2+} which react with the carboxylic groups on the SA, forming an intercellular gel matrix. The gel formation mechanism is mainly named the “egg-box” model, and in this process the bivalent cations are chelated by oppositely-charged G sequences following coordinate interactions that lead to such specific arrangements, growing into multimers by lateral associations.³⁵ SA hydrogels have numerous applications in the industry as a gelling and encapsulating agent, but despite their advantageous features, hydrogels made with SA alone display some disadvantages like high swelling rate and poor mechanical properties,³⁶ slow responsiveness to triggering stimuli, possible drug deactivation by drug–polymer binding, non-specific drug release under diffusion-controlled release, and furthermore SA is not enzymatically broken-down in mammals causing poorly regulated degradation.^{37,38} To overcome this, the use of composite crosslinkers leading to covalent modifications between SA with organic or inorganic materials have resulted in more promising functionalities.^{36,39,40}

Alginate bead gels are usually molded by pouring dropwise an aqueous dispersion of alginate into an aqueous solution of Ca^{2+} , and through the α -gulonate (DGL) and residues of SA, DGL entraps divalent cations and forms a stable, continuous, and thermo-irreversible 3-D network with the typical “egg-box” conformation while forming more rigid structures.⁴¹ The crosslinking reaction rate varies depending on the type of divalent salt used; a fast gelation could be reached when $CaCl_2$ is used, nevertheless, the

denseness for the crosslinking reaction as well as the alginate concentration gradient within the gelled bead is not homogeneous. Conversely, the use of CaCO_3 , which is less soluble in water, achieves a more uniform distribution in the alginate solution before gelation occurs.⁴² Different crosslinking processes require the addition of organic copolymers, like polyvinyl alcohol, or organic crosslinkers like glutaraldehyde⁴³ and thioglycolic acid,⁴⁴ aiming to reinforce the structure of providing new functionalities.

2.3.1.2 *Hydrogels Formed by Interpolymer Complexes*

A different way of using alginate in drug carrier systems is through its complexation with organic polymers or inorganic materials. The resultant hydrogels improve the functional properties of pure alginate. They are referred to as hybrid hydrogel systems (HybHS) because they consist of the formation of interpenetrating (IPN) or semi-interpenetrating (S-IPN) networks, due to the presence of two or more crosslinked networks at the molecular scale. In this way, recently Gong *et al.*⁴⁵ first developed an alginate HybHS, and their synthesis included the use of dopamine-grafted oxidized alginate–polyacrylamide;⁴⁶ crosslinked collagen peptide-functionalized with carboxymethyl chitosan and oxidized methacrylate SA;⁴⁷ calcium silicate–alginate composite;⁴⁸ silk fibroin and alginate crosslinked *via* emulsification with CaCl_2 and glutaraldehyde;⁴⁹ gliadin/alginate coacervate under crosslinking of transglutaminase (TG) and Ca^{2+} ions;⁵⁰ and SA-copolymers of methyl vinyl ether and maleic anhydride.⁵¹ The addition of these organic or inorganic materials contributes to a better hydrogel structuring, compared to those made with pure alginate, enhancing the mechanical and rheological properties, controlling the absorption of water and syneresis, roughness, porosity, and allowing the design of tunable drug delivery systems through the application of specific stimuli like pH, temperature, ionic strength and mechanical or shear stress.

2.3.1.3 *Micelle-based Hydrogels*

Biopolymeric micelles are systems formed by self-assembling from amphiphilic blocks or graft copolymers, and these systems have demonstrated controlled release, drug targeting, and hydrophobic drug

solubilization activities.⁵² They have been used to overcome some disadvantages exhibited by the core-shell hydrogel conventional systems, which are prompted to break when submitted to high-shearing applications. Alginate by itself does not have the property for self-assembly and micelle formation, because it does not have sufficient amphiphilicity for it. Macromolecules like hydrophilic polymers have been chemically modified to transform them into more hydrophobic molecules, providing new functionalities like micelle self-assembling structures, and these modifications can be easily formed by chemical crosslinking reactions or by UV irradiation.⁵³ The long-term stability of these micelle systems can be tuned by the structure and composition of the molecule.⁴⁶ Dai *et al.*⁵⁴ reported the use of a propylene glycol-alginate derivative, an amphiphilic biopolymer obtained by the esterification of alginic acid with propylene oxide, while Yang *et al.*⁵⁵ synthesized an octyl-grafted-alginate-amide derivative for modifying the alginate hydrophobicity, and their findings indicate that chemical grafting in alginate led to obtaining better surface activity and improved emulsifier and foaming behaviors. Fang *et al.*⁵⁶ reported the grafting for the synthesis of amphiphilic alginate derivatives by a four-component condensation reaction: alginate-formaldehyde-cyclohexyl isocyanide-octylamine, and this modification increased the hydrophobicity in the alginate molecule, exhibited higher amphiphilicity, and observed the self-assembly of the grafted alginate into micelles.

2.3.1.4 Emulsion Gels

Emulsion gels are considered a kind of soft-solid materials where the droplets from the emulsion are inserted into the network of crosslinked polymers, or in shape of a network formed from flocculated emulsion droplets.⁵⁷ They combine the functional properties of both emulsions and hydrogels and are categorized as droplet-filled-gel or droplet-aggregated-gel.⁵⁸ The final configuration depends on the interactions carried out among the components in the emulsion and the gelation mechanisms, resulting in more complex systems. The 3D network structure in the emulsion gels give the oil phase with improved rheological and texture properties,⁵⁹ and make that oil droplets act as inactive fillers when there is null interaction with the gelling continuous phase, or as active fillers when the interactions occurred

between phases modifying the rigidity of the gelled phase or the interfacial properties for the emulsifier layers that surround the oil droplets, switching the active fillers into inactive fillers, and *vice versa*.⁵⁷ This structuration process permits minimizing the diffusion of bioactive entities encapsulated in the core phase, promoting the protection of active compounds from free radical oxidation induced during manufacture and storage,^{60,61} and controlling and sustaining its release and delivery.⁶² Emulsion gels often exhibit physical and rheological properties comparable to those displayed by hydrogels, with clear effects from factors like oil type, mass or volume dispersed fraction, the gelling matrix, and interactions between components.⁶³ Moreover, the gelled phase could also be formed by composite matrices, which produce great diversity in gel structuration like (1) a gelling phase containing a great number of dispersed fluid parts; (2) a gelled phase trapped within a foremost fluid phase; (3) all phases are gelled.⁶⁴ Among the most relevant characteristics of emulsion gels are the mechanical properties, which relate to the structure of emulsion gels and their behaving to deformation, and break-down attributes, which allows the design of more easily broken-down gels at small deformations.⁶⁵

2.3.1.5 *Aerogels*

These systems are defined as nanosized porous materials with large volume, pores, and surface area.⁶⁶ They are formed like particles, fibers, thin films, and monoliths. Among their most outstanding characteristics are their good transparency, flexibility, high porosity, low weight and thermal conductivity, and excellent mechanical strength, making them suitable materials for a great variety of applications in pharmaceutical and biomedical topics. Aerogels are obtained in various sizes and shapes, and their formation is achieved by using a sol–gel procedure, followed by removing the liquid phase by freeze-drying or supercritical CO₂ drying techniques, preserving the gel structure but maximizing the surface area^{66,67} becoming excellent drug carriers. These properties enhance the inner and outer functionalization by coating, and/or encapsulating them with other polymers, setting better functional properties.⁶⁸

2.3.1.6 *Hydrogel Films*

Hydrogel films are commonly utilized as coating materials, they have been considered key elements for appliances joined with biological approaches. Achieving the appropriate processing conditions that will maintain the biocompatibility and integrity of encapsulated core materials has resulted in great challenges during the design and production of these polymer matrices.⁶⁹ Alginate-based hydrogel films have been used applying different reinforcement methods, like the formation of IPNs, the increase of crosslinker content,⁷⁰ the utilization of surface polymerization onto lipophilic substrates *via* plasma synthesis,⁷¹ and nanotechnologies for graphene oxide reinforcements,⁷² and carbon nanofibers.⁷³ These modifications resulted in the improvement of the mechanical and optical properties of the films, while maintaining the biocompatibility and water sorption properties.

2.3.1.7 Composite Nanoparticles

Nanoparticles (NPs) are systems used as transporters for drugs. They are characterized by having particle sizes between 10 and 1000 nm; their size facilitates their effective penetration through cell walls, offering advantages like protection of drug molecules, minimal or no toxicity, and controlled release.⁷⁴ NPs are increasingly combined with hydrogels to form hybrid biomaterials.⁷⁵ This increased their structural diversity and enhanced their drug delivery properties. The strategies for producing these hybrid systems are varied, depending on the functional activities pursued. Some examples include the maleimide-chitosan-catechol-alginate NPs (M-CS-Catch-Alg), formed by ionic gelation of Catch-Alg into M-CS with a magnetic stirrer followed by ultrasound for producing mucoadhesive systems;⁷⁴ Au/polyaniline boronic acid/SA NPs for antimicrobial activity;⁷⁶ Ag-doped biphasic calcium phosphate/SA for antibacterial and controlled doxorubicin delivery systems,⁷⁷ and chitosan/Ca-Alg NPs/AgNPs for antibacterial activity,⁷⁸ or to design sensible drug delivery hydrogels from grafted alginate/SiO₂ nanosized carriers,⁷⁹ among others.

2.4 Alginate-based Carrier Applications in Delivery Systems and Biomedicine

Drug delivery, tissue engineering and wound healing are some biomedical applications where alginate has been used in different colloidal forms such as hydrogels, films, emulsion gels, micro, and nanocarriers, *etc.* (alone or in composite systems) ([Figure 2.2](#)).

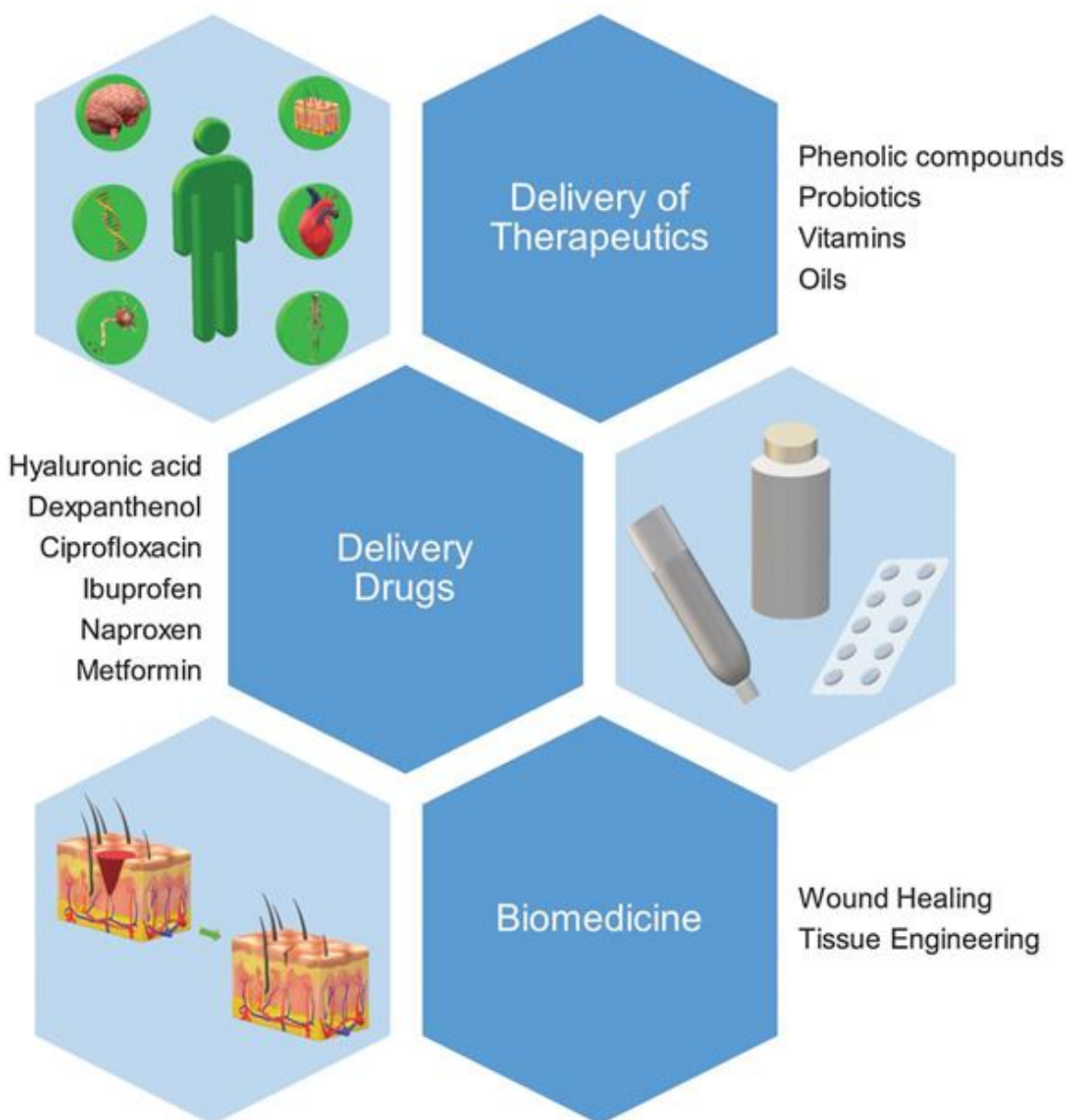


Figure 2.2 Alginate-based carrier applications in therapeutics and drug delivery systems, and in biomedicine.

2.4.1 Therapeutic Applications

Out of the variety of modified systems produced for substance delivery, complexes based on biopolymers offer several advantages over some carrier platforms due to their versatility for tailoring the carrier properties to meet specific needs, ease production, for high encapsulation efficiency, drug stability, physicochemical flexibility, to enhance polymer degradation, and for controlled release due to stimuli-responsive polymer erosion and delivery of drugs.^{80–83} In this sense, films, bulk hydrogels, microcarriers, and nanocarriers, have been developed from natural polymers like alginate, chitosan, gum Arabic, and carrageenan, among others;^{84–88} their structural properties allow them to be used as film formers, emulsifiers, stabilizers, thickeners, and gelation agents.^{4,89} Alginates have antimicrobial and anticarcinogenic properties, and have demonstrated beneficial effects on human health through blood pressure and cholesterol reduction as well as diabetes and adiposity prevention; additionally, their biocompatibility, non-toxicity, biodegradability, low cost, and easy handling make this polysaccharide attractive as a biological ingredient.^{4,82,83,89} One of the restraints of alginate gels is their porous structure because this leads to fast and easy diffusion which, at the same time, provokes a low encapsulation efficiency and enhances the possibility of bioactive-atmospheric oxygen contact. To solve this issue, structure modification and porosity reduction by other polymers, or coating with other substances have been realized to improve alginate performances.^{84,90}

Alginate-based systems are recognized for their stability in hot and cold environments,⁹¹ and their simplest applications are probably those focused on preventive therapeutics, where alginate is used as a carrier to control the delivery of bioactive compounds like phenolic compounds, probiotics, vitamins, and lipophilic antioxidants which contribute to better functioning of the human body. The importance of studying these bioactive compound carriers is due to their vulnerability to environmental conditions which has led to a constant search for improved biomaterials to be applied as carriers.

Phenolic compounds are formed by aromatic rings and hydroxyl groups and sometimes they contain functional groups; in plants, their main role is protection, but they are also flavor and appearance contributors. In humans, their consumption is primarily from food (fruits, vegetables, cereals, *etc.*) or

medicinal herbs (roots and leaves), and due to their antiviral, antioxidant, anti-inflammatory, and antiallergenic properties, they are associated with the prevention of illnesses like cancer, diabetes, obesity, and neural and cardiac disorders.^{84,90,92–94}

Probiotics are live microorganisms that mainly reduce damaging bacteria in the intestine aiding gastrointestinal health. However they are also related to immune defense system enhancement, prevention of colon cancer and urinary infections, reduction of allergy damage, and treatment of rheumatoid arthritis; alginate is commonly used to immobilize or encapsulate probiotic bacteria mainly due to its stability under acidic conditions.^{95–97}

Vitamins are known to be essential for human body performance, and any deficiencies can cause different disorders because vitamins are responsible for the development of several functions, for example, the respiration process needs vitamin B₂, vitamin B₁₂ is necessary for nerve health, and vitamin A is crucial for the visual system; adequate amounts of this organic compound cannot be produced by the human body so we need to obtain them from food items.⁹⁸

Essential oils are compounds extracted from herbs, fruits, trees, *etc.* that throughout history have been used for their therapeutic effects because they have demonstrated several useful properties for our physical wellness, such as antibacterial, antifungal, anticancer, antiviral, and anti-inflammatory properties; the problem with these substances is that they are not synthesized by humans, thus people need to ingest food which contains them.^{87,99}

Due to all the benefits that we can obtain from the bioactive compounds mentioned above, there has been increasing interest from investigators to explore different ways of protecting them and alginate-based systems are part of the solution to this issue. In Table 2.3 the main results obtained from the latest therapeutic application experiments for alginate-based systems are presented.^{84,86,87,90,91,93–96,100–114}

Table 2.3 Therapeutic applications.

Alginate-based system	Objective/benefit	Results/improvements	Ref.
Sodium–alginate solution	Protective effect in esophageal	Significantly longer cohesion time than a	Sonmez <i>et al.</i> ¹⁰⁰

	disease for topical drug delivery	placebo or saline-sucralose solution on <i>in vivo</i> study	
<i>Bletilla Striata</i> mixed with sodium alginate microspheres	Enhancement of adhesion to gastrointestinal tract from microspheres and wound healing, antiulcer, immune-modulatory and anti-fibrosis activities provided by the <i>Bletilla Striata</i> Polysaccharides (BSP)	BSP increased flexibility and reduced rigidity of microspheres. It also obtained a longer residence time in the stomach than isolated sodium alginate microspheres	Wu <i>et al.</i> ¹⁰¹
<i>Viola odorata</i> Linn extract microencapsulated into alginate with chitosan coating	Oxidative stability for microcapsules loaded with a sweet violet extract which contains flavonoids capable of protecting against various diseases due to its antioxidant and anti-inflammatory properties among others	Alginate–chitosan matrix showed retention ability of antioxidant compounds from the extract and a controlled release was also obtained on <i>in vitro</i> studies	Yousefi <i>et al.</i> ⁸⁴
<i>Laurus nobilis</i> L. (bay leaf) extract	Protection of polyphenols	Longer release time (15 min) than the	Chaumun <i>et al.</i> ⁹³

microencapsulated with sodium alginate	contained in the extract, which have antioxidant, anti-inflammatory, and antiviral properties	extract microencapsulated using arabic gum (6 min) or modified chitosan (9 min)	
Cake added with red onion peel extract nano encapsulated in sodium alginate	Protection of anthocyanins and antioxidants contained into the extract to help in losing weight, diminishing the risk of cancer, diabetes, and cardiovascular diseases, and also to provide antimicrobial and anti-tumor properties.	Enhancement in antioxidant activity efficiency during baking, lower blood sugar levels, and body weight reduction of diabetic rats tested.	Mahmoud <i>et al.</i> ⁹⁴
Resveratrol into sodium alginate-shelled soy protein isolate (SPI) nanoparticles	Protection of resveratrol which is a polyphenolic compound that helps with cancer, inflammation, neuron, liver, and heart disorders	Good stability under gastric conditions	Zhang <i>et al.</i> ¹⁰²
Beet green extract encapsulated into alginate beads	Stability and release evaluation of alginate beads containing beet green extract which include	Low release under gastric conditions but high release under intestinal conditions	Gorbunova <i>et al.</i> ¹⁰³

	betalains and phenolic compounds on its composition that are antioxidant substances		
Curcumin encapsulated into sodium alginate	Controlled release of curcumin microencapsulated, this compound has anti-inflammatory, anticancer, antiviral, antifungal, and antibacterial activities	Sodium alginate microcapsules showed a higher total release time of curcumin than chitosan particles but less than those formed with arabic gum	Lucas <i>et al.</i> ¹⁰⁴
Curcumin nanoemulsions into alginate hydrogel beads	Curcumin has antioxidant, antibacterial, and anti-tumor effects thus, there is a need to encapsulate and release this compound into the body	There was a great encapsulation efficiency (<99%) and the release of curcumin was lower at pH 7 than at pH 9	Xu <i>et al.</i> ¹⁰⁵
<i>Bifidobacterium animalis</i> encapsulated in Agave Fructan-Canola Oil-Sodium Alginate coacervates	Ensure transportation of the probiotic through the gastrointestinal tract to maintain its bactericidal effect	High survival rates of probiotics after simulated gastrointestinal conditions compared to free cells	Juárez-Trujillo <i>et al.</i> ⁹⁵

Beads of <i>Lactobacillus acidophilus</i> encapsulated into sodium alginate	Provide stability to beads containing the probiotics for further consumption to supply its gastrointestinal benefits to humans	Beads using alginate brought major protection than carrageenan beads to probiotics under simulated gastrointestinal and thermal conditions on <i>in vitro</i> analysis	Afzaal <i>et al.</i> ¹⁰⁶
<i>L. casei</i> entrapped into magnesium hydroxide loaded-calcium alginate beads	Viability evaluation of probiotics when beads were subjected to gastric conditions	The major viability of <i>L. casei</i> exposed to gastric conditions was obtained when 0.1 g Mg(OH) ₂ /100 g alginate concentration was added, higher content of magnesium produced gels more flexible, and probiotic cell damage	Arenales-Sierra <i>et al.</i> ⁹⁶
Lycopene from tomato pulp emulsified with olive oil or corn oil and sodium alginate	Promote stability of lycopene to preserve its healthy properties including the prevention of chronic diseases	A little reduction of lycopene photodegradation was observed when the sodium alginate was added as well as an increase of <i>in vitro</i> bioaccessibility, which was compared to results obtained from emulsions prepared with whey	Liang <i>et al.</i> ¹⁰⁷

Lycopene concentrates from watermelon into sodium alginate beads	Stabilize lycopene beads to further application as natural additives; degenerative diseases and cancer prevention are some of the lycopene benefits	protein isolated as a biopolymer Higher lycopene retention, more spherical beads, and better stability during storage were obtained from lycopene–alginate beads compared with lycopene–pectin beads	Sampaio <i>et al.</i> ⁹⁰
Vitamin D3 encapsulated in Sodium Alginate–Ovalbumin–Pectin complex	Protection and release of Vitamin D3 that has immunological benefits.	Systems containing sodium alginate show better stability and sustained delivery of active compound in the simulated gastrointestinal conditions	Gao <i>et al.</i> ¹⁰⁸
Calcium and vitamin D3 microencapsulated into sodium alginate	Bioavailability study of calcium and vitamin D3 under simulated gastrointestinal conditions; both compounds provide health benefits such as hormone and enzyme secretion, and osteoporosis and cardiovascular	Sodium alginate provides more bioaccessibility of the vitamin over chitosan microparticles	Dima & Dima ⁸⁶

	disease prevention.		
Folic acid (vitamin B ₉) encapsulated into a copper–alginate hydrogel	Evaluate stability of the copper–alginate hydrogel when folic acid is encapsulated into it to bring an alternative of supply for this bioactive compound that is essential during gestation and childhood for DNA, amino acid, and nucleoprotein construction, and is also helpful for cancer, cardiovascular, Alzheimer's, and anemia diseases.	There was no release of folic acid under simulated gastric conditions, which means great protection that allowed delivery of bioactive compounds into the intestine (basic conditions), indicating the possibility of an adequate absorption of folic acid into the human body	Camacho <i>et al.</i> ⁹¹
Cinnamon essential oil microencapsulated into whey protein isolated-maltodextrin–sodium alginate	Protection of cinnamon essential oil against oxidation, this compound has antimicrobial, antioxidant, anti-inflammatory, and anticancer properties	Improvement of cinnamon essential oil properties was observed when sodium alginate was added, loss of volatiles was diminished and oxidative stability during storage was enhanced compared to a maltodextrin–	Hu <i>et al.</i> ⁸⁷

Microcapsules of sachainchi oil (<i>Plukenetia volubilis</i> L.) using ovalbumin and sodium alginate coacervates	Develop an <i>in vitro</i> study with microcapsules to evaluate the protective effect of the matrix on sachainchi oil which is related to the prevention of some diseases, for example, diabetes and hypertension, due to its content of omega-3 on vitamin E	arabic gum encapsulating matrix A resistance to simulated gastric conditions was observed, leading to higher availability of omega-3 into the intestine	da Silva Soares <i>et al.</i> ¹⁰⁹
Sesame oil encapsulated into sodium alginate–nopal mucilage beads	Improve stability of sesame oil contained into beads to preserve its health benefits such as anti-inflammatory and antimutagenic properties	The evaluation showed higher encapsulation efficiency, better protection to oil oxidation, and slower release of the bioactive compound when nopal mucilage was present into the matrix	Velázquez-Gutiérrez <i>et al.</i> ¹¹⁰
Thyme (<i>Thymus vulgaris</i>) oil into alginate–soy protein isolate beads	Transport essential oil to its intestine release, thyme essential oil has cough, muscle tension,	A lower thyme oil release rate was obtained when the alginate concentration increased, and oil	Volić <i>et al.</i> ¹¹¹

	helminthiasis, and diuretic beneficial effects	content was mainly delivered under simulated intestinal conditions	
Ethanol extract of propolis microencapsulated into sodium alginate	Microencapsulate propolis to further consumption because of its content of essential oils and phenolic compounds that have effects against oxidation, microbial, inflammation, and tumoral activity	From an <i>in vitro</i> study, a higher percentage of propolis release was observed under simulated intestinal than simulated gastric conditions	Keskin <i>et al.</i> ¹¹²
AvrA protein nanoparticles encapsulated into alginate–chitosan microparticles	Protection of AvrA protein nanoparticles because they have displayed advantageous effects on chronic gastrointestinal inflammatory disorders	Alginate–chitosan system was effective to protect protein nanoparticles through gastric fluids, from <i>in vitro</i> and <i>in vivo</i> studies, it was observed a reduction in colitis clinical symptoms in a murine model	Ling <i>et al.</i> ¹¹³
Bovin lactoferrin–sodium alginate complex coacervates	Protection of lactoferrin structure to maintain its human health benefits such as	An increment of 30% on antioxidant capacity was obtained from the alginate matrix	Wang <i>et al.</i> ¹¹⁴

cell growth modulation and inhibition of toxic compounds formation	under simulated gastric conditions
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As shown in [Table 2.3](#), the formation of hydrogel beads is the most common system for these applications, as the ionotropic mechanism is the favorite method to prepare them and calcium chloride is the preferred solution to produce the beads. It is worth mentioning that alginate has shown better results in studies when compared to other biopolymer matrices. Additionally, research results have confirmed that the addition of other biopolymers to alginate-based systems has resulted in better performances. In general terms, alginate is relevant for bioactive compound entrapment and delivery, and even with the distinct experimental conditions used in these research studies, alginate-based systems exhibited positive results on stability under simulated gastric conditions and during storage despite a constant release of bioactive compounds under simulated intestinal conditions and good protection against deterioration of encapsulated substances. According to this information, we want to highlight the importance of alginate in therapeutic applications because, although there are still many topics to investigate, alginate-based systems have displayed great potential in this field.

2.4.2 Drug-controlled Release Systems

Dosage formulas for drug delivery seek to achieve a prolonged therapeutic effect at a low dosage rate, in addition to targeting the drug toward specific sites, which represents a challenge for the pharmaceutical industry. Alginate polymers can be processed to meet the specific requirements of pharmaceutical and biomedical industries, with a great variety of forms like hydrogels, beads, micelles, films, microparticles, or complex nanoparticles. This section highlights the most important findings for the application of different dosage formulations of alginate matrices in pharmaceutical applications ([Figure 2.3](#)), with a particular focus on the correlation of the colloidal system featured and the drug released for a specific treatment.

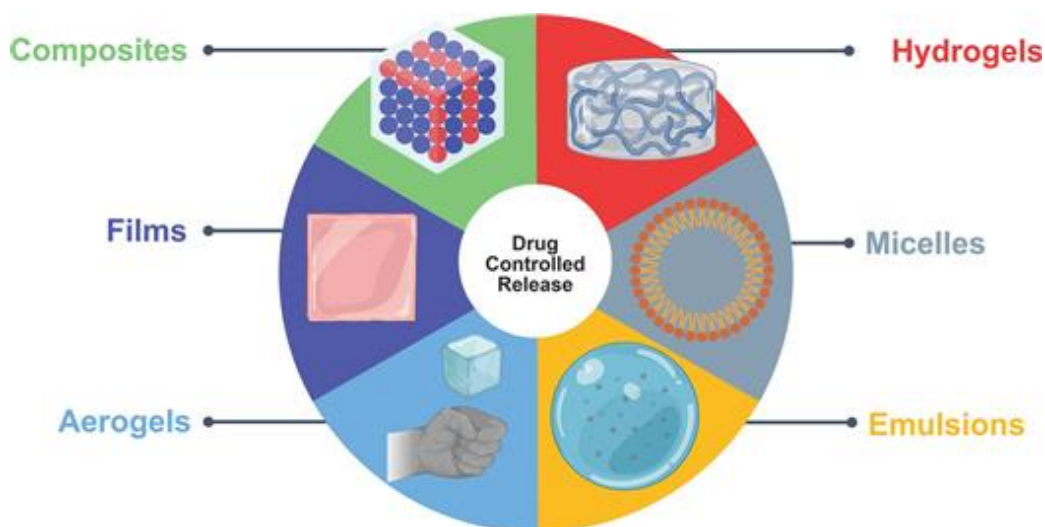


Figure 2.3 Alginate in different colloidal systems.

2.4.2.1 *Hydrogels*

Therapeutic and pharmaceutical application of alginate matrices has emerged as one of the most important uses in the release of drugs. Hydrogels are some of the most attractive matrices for drug release, which is attributed to their characteristics, for example, great water retention capacity, changeable swelling behavior, high stability, prolonged shelf life, as well as molecular arrangements. Alginate hydrogel-based systems exert a high degree of influence on the transport mechanism to release the drug with unique physical and chemical properties, and on drug preservation against environmental factors such as temperature, pH, pressure, and moisture. The water introduction inside the polymeric system contributes to the hydrogel swelling, making up a diffusion barrier that decreases the migration of small molecules.

2.4.2.1.1 *Hydrogels for Wound Dressing and Tissue Regeneration*

Alginate hydrogels are materials that have been successfully used for wound dressing (WD) and tissue engineering applications, and they are characterized by performing functions in the skin to treat wounds in the short or long term as required. A SA-Pluronic F127 hydrogel loaded with antimicrobial and antifibrinolytic agents resulted in a suitable sustained drug

release system showing good biocompatibility and cell–material interaction, maintaining the moist wound surface.¹¹⁵

WDs formed with alginate-based composite hydrogels have been evaluated as controlled release systems of drugs. In this sense, WDs with antibacterial properties and controlled drug release formed from hydroxyethylacryl chitosan–alginate hydrogels loaded with silver nanoparticles showed functionality, dependence on the crosslinking density, increment in the swelling, and delayed drug release.¹¹⁶ SA–polyvinyl alcohol (PVA) enriched with *E. purpurea* extract exhibited behaviors such as a controlled release of extract since the drug concentration was maintained for a long time to achieve its full functionality, and it is worth mentioning that burst effects were observed during the release.¹¹⁷ On the other hand, biocompatible PVA–SA hydrogels loaded with dexpanthenol, used as the core material in chitosan-nanofiber WDs, exhibited Fickian diffusion mechanisms for drug delivery, mainly controlled by the chemical potential gradient and swelling of the coaxial nanofibers.¹¹⁸ George *et al.*¹¹⁹ produced injectable hydrogels from PVA–SA loaded with vitamin C nanoparticles, and *ex vivo* permeation studies showed that the active compound is capable of completely inhibiting *S. aureus* bacteria because it can diffuse through L929 cells, showed biocompatibility, and was released in a sustained manner, demonstrating its usefulness to treat skin and soft tissue infections.

An alginate–gelatin–hydroxyapatite IPN composite exhibited better control over the burst release of the ciprofloxacin drug model, prolonging and sustaining its release from 5 to 240 h, and displaying great antibacterial activity.¹²⁰

In situ hydrogels composed of alginate and chitosan were loaded with sodium diclofenac drug (anti-inflammatory) to be applied by intra-articular *via*. The preparation consisted in forming alginate microspheres loaded with the drug by the gelation method, and later were dispersed inside of thermosensitive hydrogels in injectable form, consisting of chitosan and β -glycerophosphate. The anti-inflammation effectiveness of the blended hydrogels over rheumatoid arthritis demonstrated that these hydrogels improve the therapeutic effect of diclofenac sodium for intra-articular administration.¹²¹

2.4.2.1.2 Hydrogel Beads (For Oral or Colon Administration)

The most popular way to produce alginate beads to encapsulate different drugs is by a technology known as alginate gelation. The encapsulation of substances aims to protect and provide functional properties. Alginate is a raw material widely used as a wall material in encapsulation and can form beads with multiple structural arrangements.¹²² Beads made for drug delivery offer advantages as the drug transportation to the specific site lowering the biological impact on the host.

SA beads were loaded with a self-micro-emulsifying drug delivery system of hesperidin, a drug with antioxidant, diuretic, anti-inflammatory, antidiabetic, anticancer, vasoprotective, and cholesterol-lowering actions. The *in vitro* hesperidin release was shown to protect the hesperidin in the gastric environment and had pH-dependent burst release in intestinal conditions.¹²³

The Fe^{3+} -crosslinked sodium alginate–methylcellulose semi-interpenetrating beads loaded with ibuprofen were formulated to be administered orally. Ibuprofen is a non-steroidal drug that has mainly anti-inflammatory and analgesic functions. The optimum drug release of 93.9% for 6 h was obtained using 66.7% (w/w) of SA and 33.3% of methylcellulose, with a 1 : 4 drug–polymer ratio, 0.1 M of FeCl_3 , and 90 min of crosslinking time for the bead preparation. The release mechanisms of ibuprofen from beads that had a better fit were the anomalous and the case II.¹²⁴

Environment-sensitive alginate hydrogels were formulated as drug delivery vehicles through the Diels–Alder reaction. Alginate was functionalized with furfurylamine (FUR) *via* amidation, and the reaction was carried out using poly-propylene oxide-*b*-poly(ethylene oxide)-*b*-poly(propylene oxide) bismaleimide which was employed as a crosslinker. This strategy generates pores more homogenous with more interconnected porous structures, improving the physical characteristics of the crosslinked alginate network and acquiring more sensitivity toward environmental stimuli, and improving drug delivery.¹²⁵

SA was mixed with basil seed mucilage (BSM) for forming beads loaded with metformin, a drug which lowers blood glucose levels to treat type 2 diabetes disease. The addition of BSM and sodium tripolyphosphate (cross-

linker) to SA decreased the drug release rate. The metformin release was pH-sensitive and showed a higher release rate at pH 7.4.¹²⁶

The alginate formed a complex with divalent copper for folic acid encapsulation in hydrogel beads. The acid folic (vitamin B₆) is highly recommended during pregnancy as its function is to prevent neural tube defects throughout the development of the fetus. Thus, copper alginate as an encapsulant achieved stability and slow folic acid release under simulated physiological conditions.⁹¹

The alginate was modified with sodium dodecyl sulfate (SDS) for use as an encapsulant material of drugs whose compositions are made up of proteins, in this case, bovine serum albumin was chosen as a drug model. The release study showed that under gastric conditions (pH = 1.2), the modified gel reduced the protein release rate regarding the unmodified gel, and under intestinal conditions, the drug release time from the modified gel was prolonged due to an increase in the stiffness of the material. The SDS-modified alginate beads avoid drug degradation through the gastrointestinal tract to the colon.¹²⁷

SA was chemically modified to produce thiolated sodium alginate (TSA) through esterification with thioglycolic acid. SA and TSA microspheres in different proportions were loaded with metformin hydrochloride. The TSA improved the mucoadhesive properties and had the greatest control release effect of metformin (pH = 6.8) which is beneficial in providing a prolonged therapeutic effect. SA–TSA microspheres containing metformin were evaluated for their drug release, the results showed a non-Fickian-type release behavior, and were properly fitted to the Korsmeyer–Peppas release model.⁴⁴

SA and sericin (a protein) were used individually and in mixture, along with cross-linkers like sodium phosphate dibasic (Na₂HPO₄), polyvinyl alcohol (PVA), and polyethylene glycol for loading naproxen (a nonsteroidal anti-inflammatory drug). Using a simulated gastric fluid (SGF) at pH = 7.4, the delayed release of the drug was determined, which in one case lasted up to 360 min, demonstrating gastro-resistant behavior, besides, covalent crosslinking had no significant effect. The release mechanism for the alginate–sericin blend was described by swelling and polymer chain relaxation.¹²⁸

SA was mixed with a dispersion of ZnO nanoparticles (ZnO NPs) stabilized using carboxymethyl chitosan (CMCS) to control the diclofenac sodium (DS) release from hydrogel beads. DS is a non-steroidal anti-inflammatory, antipyretic, and analgesic drug. *In vitro* release studies showed that CMCS–ZnO NPs incorporation to SA helped to reduce the drug release rate in simulated gastric and intestinal fluids to avoid irritation. *In vivo* studies showed that there was higher bioavailability and slower release of DS using the mixture of SA/CMCS–ZnO hydrogel beads after oral administration.¹²⁹

Oral colonic delivery has also been analyzed with the use of liposomes loaded with capsaicin encapsulated in alginate hydrogel beads. For the liposomes' preparation, soy lecithin and cholesterol were used and the procedure to achieve it was thin-film hydration, then, the encapsulation of the liposomes was carried out by ionotropic gelation where the beads were coated with Eudragit. The results showed an amount of drug coming into the colon was greater than 65%, demonstrating its potential for use as a carrier for colonic drug delivery.¹³⁰

Porous cores have also been developed using alginate–chitosan-based nanoparticles for oral delivery of an anticancer drug. This work consisted in the formation of multilayer beads, porous and non-porous, by the ionic gelation method. The results of the porous beads had a higher entrapment efficiency and slower drug release in the gastrointestinal tract. On the other hand, it was shown that the anticancer drug (doxorubicin hydrochloride) can be administrated orally, and its bioavailability is guaranteed because the beads were resistant to the acidity of the gastric fluid since they did not release a considerable amount of the active compound.¹³¹

Liposomes containing drugs have been encapsulated in microspheres of hydrogels as a new drug delivery system. An example of liposome microbead formation was performed using as encapsulating materials: alginate, alginate–sucrose, and alginate–chitosan which achieved the protection of resveratrol liposomes.¹³²

A hybrid carrier agent for colon drug delivery conformed by guar gum succinate–sodium alginate was prepared as a pH-sensitive delivery product. It was reported that the hybrid beads did not show apparent cytotoxicity and it was concluded that these could be safe for oral administration to deliver the drug in the colon in a more specific way.¹³³

The targeted release has also been explored by magnetic control, and in this way, Kondaveeti *et al.*¹³⁴ developed magnetic responsive alginate/magnetite hybrid beads to release dopamine. This model pretends to be valid for administering any type of drug with the condition that the drug and matrix are electrostatically attracted, however, to validate this model *in vivo* studies will have to be carried out to guarantee its safety by complying with the regulation of the drugs.

Another system of Eudragit coated alginate beads was used to protect liposomes composed of folic acid and L-OHP drug with the aim of reducing the dosing frequency of the active compound and improving the chemotherapy in the treatment of colorectal cancer.¹³⁵

Core-shell formulations of alginate hydrogels were tested to reduce gastric acid secretion by the delivery of ranitidine HCl and aceclofenac. Microbeads of alginate hydrogels were loaded with aceclofenac coated with Eudragit L-100[®] and chitosan-PVA gels containing ranitidine HCl. The release of ranitidine HCl followed the super case-II transport mechanism in both media, while the release of aceclofenac was carried out by anomalous diffusion.¹³⁶

The technology of prilling by ionic gelation was used to develop Zn-alginate beads containing ketoprofen for the treatment of inflammatory-based diseases. This work reported that *in vivo* Zn-alginate beads had a prolonged anti-inflammatory effect, compared to pure ketoprofen, with significant anti-inflammatory activity.¹³⁷

Alginate beads loaded with self-emulsifying curcumin achieved crossing the upper gastrointestinal tract without allowing the release of the drug, therefore, they were able to specifically reach the colon where they were released to fulfill their function.¹³⁸ This technique was also used for the release of Captopril controlled release which was achieved by a controlled swelling from alginate beads using galactomannan with Senna Tora, guar, and locust bean gums.¹³⁹ Calcium alginate beads loaded with Celecoxib drug have also been analyzed,¹⁴⁰ and these were dissolved in a self-emulsifying phase to ensure drug release on site. The hardening treatment using calcium chloride solution of 100 mM for 15 min and a high concentration of the drug improved the mechanical resistance of the beads, generated less swelling, and resulted in adequate drug release in the intestine.

A cocrystal of chitosan was attached into alginate beads for better release of aceclofenac using an ion-gelation technique, to obtain a new drug release method called the supersaturated drug delivery system (SDDS). This research found that the coating of alginate over the aceclofenac drug in the beads helps in controlling the release and prolongs the absorption of the drug, thus this SDDS is adequate in the oral bioavailability of the drug.¹⁴¹

2.4.2.2 *Films, Fibers, and Particles*

Alginate and gellan gum were used to produce microparticles, films, and oral tablets of morin. Morin has activity against cariogenic microorganisms. The *in vitro* morin release was carried out using artificial saliva and it was demonstrated that microparticles with 39.6% of morin release achieved greater control over the drug release than the film form with 41.1% and the tablet form with 91.4% of morin release after 20 h.¹⁴²

SA and feather keratin were blended to create a fiber using the wet spinning method to generate a skin-core structure. This fiber was used to carry indomethacin, a drug used in the treatment of fever and inflammation, besides, in pain symptoms such as osteoarthritis, rheumatoid arthritis, *etc.* The indomethacin release reached 80% after 12 h in colon fluid and was less than 20% in digestive fluid. It was observed that the increase of feather keratin extended the drug release time due to the hydrogen bond formation.¹⁴³

A bio-hybrid drug carrier was formed using colloidal silica nanoparticles and SA. The carrier was loaded with doxorubicin (an antitumor drug) using the spray drying technique. In an *in vitro* study, the bio-hybrid carrier allowed the drug to be released slowly and continuously which helped to maintain the drug concentration in the cells for a longer time, also, the drug release was pH-dependent showing higher cumulative drug release at pH 5.5. Thus, the bio-hybrid showed cytotoxicity to lung carcinoma A549 cell lines.¹⁴⁴

Nanoparticles of complexes were constituted using ovalbumin, pectin, sodium alginate, and vitamin D₃ through the antisolvent precipitation and the freeze-drying methods. The addition of sodium alginate improved the stability of the complex and allowed the reduction in particle size. These nanoparticles were swollen and showed a low cumulative release under

simulated gastric conditions (SGF) while losing their structures when subjected to simulated intestinal fluid (SIG) completing vitamin release. The release kinetics of the vitamin had a better fit to the Higuchi model.¹⁰⁸

2.4.3 Biomedical Applications

Tissue engineering and wound healing are biomedical applications where alginate has demonstrated potential because of its biocompatibility, biodegradability, ability to promote a moist environment, mechanical properties on biomaterial scaffolds, gel-forming capacity, and hemostatic characteristics. Commonly, alginate has been used in combination with other compounds because isolated, alginate has the disadvantages of low cell adhesion and poor stability.^{145,146}

It has been observed that wound healing treated with alginate dressing prevents dehydration due to its swelling capacity, which allows a moist medium that promotes faster wound healing since it stimulates cellular activity. SA is usually included in hemostatic materials due to its calcium ion exchange capacity for sodium ions derived from the injury exudate and this promotes coagulation.^{49,117,147}

Tissue engineering's main objectives are to repair, protect, and replace damaged tissues or organs. An important characteristic required for these applications is the presence of void spaces (porosity) which allows nutrients and oxygen to be diffused and the water holding capacity which permits cell proliferation.¹⁴⁸ In this section are shown studies that have been carried out using alginate in combination with other compounds to be applied in tissue engineering and wound treatment.

2.4.3.1 Hydrogels

Alginate hydrogels studied in recent years have shown their potential in different biomedical applications. On skin tissue engineering, hydrogels obtained using calcium carbonate mixed with glucono- δ -lactone (GDL) as a crosslinking agent to sodium alginate with vitamin D3 (3000 and 30 000 IU) solutions were tested in the animal model and it was observed that the addition of 3000 IU of the vitamin D3 into the hydrogels gave the highest results of cell proliferation rate, wound closure, capillaries production ratio, re-epithelialization degree, and collagen thickness.¹⁴⁹

Gao *et al.*⁴⁰ using the system platelet-rich-plasma (PRP) carrier, observed that PRP addition modified the gelation rate and swelling ratio of the alginate–CaCO₃–GDL hydrogel, producing a structure more homogeneous with pore sizes from 200–300 µm, and higher mechanical properties. These gels showed high cytocompatibility, supporting bone marrow-derived mesenchymal stem cell proliferation.

The addition of halloysite nanotubes (HNTs) into alginate hydrogels was demonstrated to improve their physical properties. HNTs consist of natural mineral nanotubes used mainly as polymer nanofiller because of their good dispersion properties, low toxicity, biocompatibility, low cost, and reinforcing ability for composite systems. Huang *et al.*³⁶ synthesized a columnar composite hydrogel with HNTs and SA by a casting process with CaCl₂ as the crosslinker. The addition of HNTs improved the rheological properties of SA hydrogels, controlling the swelling and water absorption processes, due to a more compact structure and the conservation of crystalline HNTs structure within the hydrogel. These hydrogels exhibited the improvement of preosteoblast (MC3T3-E1) cell culture, by increasing the cell adhesion and proliferation compared to the pure SA hydrogel.

Crisostomo *et al.*¹⁵⁰ reported the incorporation of extracellular matrix components (ECM) into alginate hydrogels, to improve the entrapment, survival, and function of beta cells which are insulin-producing cells used in the bioartificial pancreas transplantation. Tripeptide Arg–Gly–Asp (RGD) was grafted on alginate hydrogels, and their ECM contained almost a third part of the integrin receptor family and a variety of proteins including collagen type I, fibrinogen, fibronectin, laminins, and vitronectin; they showed the formation of microspheres with particle sizes around 1270 µm, where the beta cells were homogeneously distributed in the hydrogel matrix. Hydrogels prepared in this investigation had a good performance as an enhancer of beta cell insulin secretion for diabetes treatment and of *in vitro* functionality.

Alginate dialdehyde (ADA)–gelatin (G) injectable hydrogels were evaluated on a partial reconstruction of meniscal tissue and the results indicated that 15% ADA and 20% G hydrogels have potential for this application according to their gelling time and compressive strength due to its high degree of crosslinking.¹⁵¹

In tissue engineering, scaffolds are used to give support to new growth and act as a carrier and a platform to cells. For the injectable hydrogel (ADA-G), the incorporation of fibrin was carried out and the results showed hepatic cell functionality, growth, and proliferation. These hydrogels had great porosity, thus, can be used efficiently on 3D scaffold systems for liver tissue engineering.¹⁴⁵

Square propolis–sodium alginate at different concentrations utilizing calcium chloride as a crosslinker produced with a 3D printer was used to fabricate scaffolds. It was found that a combination of 4.5% (w/w) of SA and 20% (v/v) of propolis shown adequate pore size, and antimicrobial and cytotoxicity properties to be applied in tissue engineering.¹⁵² On the other hand, when methacrylate decellularized EMC from porcine bone tissue was added to alginate bioink, it was demonstrated to be a viable compound to prepare scaffolds. This composite had higher osteogenic and cellular activities than that of pure alginate, thus, it can be used in bone tissue engineering.¹⁵³

Different strategies for hybrid alginate hydrogels include the work done by Han *et al.*,⁴⁸ who synthesized a calcium silicate (bioglass BG)–alginate composite since calcium silicate is a bio-ceramic material with potential application in bone tissue engineering. The BG–SA–glucono- α -lactone (GDL) composite hydrogel showed good physicochemical and cytokinetic properties, resulting in a suitable bioactive injectable hydrogel. The appropriate combination of BG–SA–GDL develops a weak acidic medium that allows a sustained release of Ca ions from BG, developing *in situ* crosslinking of SA, which results in adequate properties for an injectable hydrogel to be obtained. In this sense, Kong *et al.*¹⁵⁴ used a BG–SA–GDL hydrogel as a basis for designing upregulating angiogenic growth factors and a revascularization facilitator system. They formed a composite with Si ions in bioglass (BG) and desferrioxamine (DFO) and alginate, and this injectable hydrogel facilitated diabetic skin wound healing at a higher rate than the control samples, where the composite components worked synergistically in promoting the vascularization in wound sites. This behavior was related to the activity of BG composed of Ca and Si ions, whose ions promote not only osteogenesis, angiogenesis, and wound healing, but also the control of hydrogel structure.

Nazarneshada *et al.*,¹⁵⁵ reported the functionality of alginate hydrogels by the addition of hydrogen sulfide (H_2S) and further crosslinking process with $CaCl_2$ (75 mM) for designing a wound dressing material. A porous structure with interconnected pores was formed, attributed to the hydrophilic–hydrophobic nature of H_2S that modifies the structure of the hydrogel. Pore sizes in the H_2S –alginate hydrogel ranged between 56–94 μm which are suitable sizes for cell infiltration and migration, and are characteristics desirable for tissue regeneration. The addition of H_2S favored the swelling properties in the hydrogel, maintaining a moist environment on the wound site, with good coverage on the wound surface, facilitating the cell migration and drug delivery, obtaining a composite hydrogel capable of absorbing the wound fluid with a wound healing efficacy of 98% using 2% of SA and 0.5% of H_2S in the hydrogel.

SA hydrogels with silk sericin were added with silver nanoparticles (Ag NPs) which increased the antibacterial activity of this hybrid hydrogel. The SA (2% w/v), silver nitrate solutions at different concentrations, calcium gluconate (0.6% w/v), and silk sericin (0.5% w/v) were mixed, and afterwards, the light exposition stimulated the Ag NP formation. The bactericidal effect of the silver NPs avoids wound infection which is important during the healing process.¹⁵⁶

Hen *et al.*⁴⁷ proposed a double-network crosslinked hydrogel formed using oxidized methacrylate–SA and carboxymethyl chitosan functionalized with collagen peptide. The composite hydrogel exhibited higher biocompatibility, an appropriate degree of swelling, a porous structure, and improved mechanical properties than conventional alginate hydrogels. A mouse model with a thickness skin defect was used to perform *in vivo* studies, and a significantly accelerated wound healing was achieved that can be attributed to inflammatory process regulation which led to vascularization improvement and collagen deposition promotion.

2.4.3.2 Emulsion Gels

Emulsion gels from alginate have been proposed as an alternative for 3D cell culture, since this conformation allows the creation of a cellular microenvironment that mimics the interactions between cells and the cellular

matrix in the body, allowing normal cell growth, gene expression, and cellular functions to be maintained.¹⁵⁷ Liu *et al.*¹⁵⁸ produced porous alginate beads with a controllable interconnected porous structure with aqueous two-phase (ATPS) emulsions as the template for 3D cell culture. These emulsions were formed with two biocompatible immiscible aqueous phases of a cell/dextran mixture and alginate/polyethylene glycol mixture and was stabilized by protein particles. The pore size of alginate beads was controlled by changing the emulsification conditions obtaining desirable characteristics, besides the encapsulation of the cells in the interconnected pores improved their activity (>95%), proliferation, and functions compared with the cells encapsulated in alginate beads.

Hemostatic microspheres were obtained by silk fibroin and alginate crosslinking *via* emulsification for wound healing. The crosslinked systems were synthesized by forming an O/W emulsion, where the silk fibroin–SA mixture was used as a stabilizer, the CaCl_2 as a crosslinker, and the glutaraldehyde for achieving the strength of the hydrogel matrix. The main characteristics observed were a rough surface that allowed adherence to the surface erythrocytes and platelets, stronger water absorption that reduced the loss of blood and tissue fluids, low *in vitro* toxicity, and an increment in the hemostatic efficiency.⁴⁹

2.4.3.3 *Films for Wound Dressings*

These kinds of films are used as a protective barrier and for promoting wound healing. In a recent study, it was found that sodium alginate-based thermosensitive hydrogel membranes enhanced wound healing due to their swelling ability, drug release, antibacterial effect, and faster reduction of the wound surface. In this research, nine samples with different SA/poloxamer 407/pluronic F127/PVA ratios were examined. The samples with higher alginate concentrations showed water vapor transmission rate values (2050 to 2296 g m^{-2} per 24 h) close to the ideal for wound dressings, which avoids their dehydration.¹⁵⁹

On the other hand, a sodium alginate–pectin hydrogel film loaded with simvastatin (drug) was tested on induced diabetic rats. SA and pectin solution (1 : 1) were dissolved before the glycerol addition, then the ethanolic solution (20 mg mL^{-1}) of the drug was added to the gel and dried

in an oven. Finally, the films were immersed in a CaCl_2 solution (0.5% w/w) to produce the crosslinked hydrogel films. The rat group treated with this film showed ~99% of wound closure after 21 days, and it was greater than the controls, besides, accelerated re-epithelialization was observed.¹⁴⁶

Bionanocomposite films of SA incorporated with distinct amounts of zinc oxide (2–10% v/v) were made using a solvent casting method and it was observed that films with 10% of zinc oxide had the highest inhibition zone and its antibacterial activity against *S. aureus* was higher than for *E. coli*, thus, these films can be used in wound treatment.¹⁶⁰

In other work, an alginate coating was deposited on a 58S bioactive glass (BG) scaffold which made it rougher and kept it on the amorphous state since the BG tends to crystallize leading to a reduction in its bioactivity, besides, the alginate addition improved the bacterial inhibition and the mechanical properties of the scaffolds used for bone healing.¹⁶¹

2.4.3.4 *Particles and Fibers*

Silk fibroin–sodium alginate microspheres were applied as hepatic arterial embolization agents for liver cancer therapy. The rabbit ear model study showed artery embolization in three weeks, excellent controlled release of Adriamycin hydrochloride (drug), and furthermore the microspheres promoted the fibroblasts growth.⁶²

Nanoparticles (NPs) of titanium dioxide–temozolomide and sprayed alginate–titanium dioxide–temozolomide were prepared, and then their effect on neuroblastoma mice was probed. Different advantages were found adding alginate to the NPs such as higher water absorption capacity, slower drug release time, better antioxidant and anti-inflammatory properties, higher curative ability, and size reduction of the neuroblastoma.¹⁶²

Fibers of SA and hyaluronic acid hydrogels were produced by two different methods. The first consisted of SA solution extrusion into CaCl_2 solution (1.5% w/v), and then the fibers were dehydrated. These dried fibers were dipped in hyaluronic acid solutions (0.25, 0.5, and 1.0% w/v) and then were dehydrated again. The second consisted of a direct extrusion of sodium alginate and hyaluronic acid solution into calcium solution. The fibers obtained from both procedures were transformed into unwoven layers and the addition of hyaluronic acid to alginate hydrogels improved its water

absorption properties, biocompatibility, and cell interaction for treating exuding wounds.¹⁴⁷

Arginine and bacitracin were mixed with a core-shell nanofibrous matrix of SA with poly-3-hydroxybutyric acid and were produced by co-axial electrospinning. The drug-controlled release, the fibrous structure, the wound contraction, the collagen deposition, and the re-epithelization were related with the alginate concentration in the nanofibrous matrix developed for its application in wound healing.¹⁶³

2.5 Conclusions

Alginate is a polysaccharide with widely explored applications ranging from food to pharmaceutical and therapeutic presentations. This polysaccharide owes its industrial potential to its surface and functional properties that allow it to perform excellent gelling, thickening, emulsifying, and stabilizing activity in dispersed systems. Various approaches have been described for the use of conventional and emerging technologies for the extraction of alginate, not only aiming to improve its yield recovery but also to enhance its functioning as a biodegradable and biocompatible surface-active material. These technologies range from the use of conventional solvent extractions to the use of enzymatic reactions, radiation, or extreme pressure conditions. Additionally, recent studies have developed alginate functionalization by chemical grafting or crosslinking, developing novel functionalities such as its increased hydrophobicity or the improvement of its surface activity, allowing alginate, a polyelectrolyte-type macromolecule, to lead to the formation of micellar structures or to achieve the formation of nanometric materials. Practical approaches to the use of alginate highlight its importance and versatility in use as a drug carrier and controlled release systems, and also allow the design of innocuous materials ranging from the treatment of superficial wounds, tissue engineering, and nanocomposites (nanoparticles and fibers) for the drug delivery to the target site achieving improved efficacy. Therefore, a better knowledge of the relationship between the physicochemical and functional properties in alginate will permit to expand the possibilities of treating various diseases and conditions, and even develop different alginate–cell and alginate–tissue interactive systems that might accomplish novel advancements in biomedicine and tissue engineering.

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Chapter 3

Alginate-based Carriers for Transdermal Drug Delivery

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3.1 Introduction

During the last two decades, transdermal drug delivery systems (TDDSs) have been extensively drawing the attention of drug-delivery researchers, and pharmaceutical and cosmetic companies as a promising drug development strategy.¹ Although a peroral route of administration has been the most preferable to date because of numerous potential advantages including painless self-medication, convenience, and patient-compliance, it has some drawbacks such as poor stability in gastro-intestinal fluid, hepatic first-pass-effect, possibility of drug-degradation by gastric acid and various

enzymes and difficulty in controlling drug-release.² Parenteral routes also have several limitations including painfulness, non-self-medication, poor patient compliance, and high cost.³ Some of these drawbacks associated with peroral and parenteral routes could be overcome by exploration of TDDSs. TDDSs can be defined as devices which are capable of delivering drugs to the deeper layers of skin as well systemic circulation through the outer layer of skin.⁴ This promising route offers several advantages over peroral and intravenous routes of drug administration including optimum efficacy, low cost, painless self-medication, patient-friendly nature, no mucosal, gut, or hepatic-first-pass metabolism, better control in drug-release, easier patient compliance, and decreased side-effects and gastrointestinal complications.¹ TDDSs also allow the use of comparatively potent drugs with minimum risk of systemic-toxicity, and both hydrophilic and hydrophobic drugs and protein or peptide drugs without the risk of gastro-intestinal degradation.^{3,5-7}

A wide range of polymers have been explored in the fabrication of TDDSs, which includes natural polymers (sodium alginate, chitosan, gum Arabic, starch, gum tragacanth, gelatin, shellac, casein, natural rubber, *etc.*), semisynthetic polymers (cellulose acetate phthalate, ethylcellulose, nitrocellulose, hydroxypropylmethylcellulose, carboxymethylcellulose), synthetic elastomers (polybutadiene, polyisoprene, polysiloxane, styrene), and synthetic polymers (polyvinyl alcohol, polyethylene, polypropylene, polystyrene, polyvinylpyrrolidone, polyvinyl chloride, polyacrylamide, *etc.*).^{8,9} The ideal transdermal matrix-forming additives should have an excellent water-absorption capacity to absorb wound exudates, protective property to prevent secondary infections, and suppressing-capacity on necrosis of a wound bed. They should permit gaseous exchange through themselves and should be biocompatible, non-toxic, elastic, non-immunogenic, non-irritating, easily removable from skin, and non-comedogenic.^{10,11} Natural polysaccharides have been widely reported to possess most of the ideal characteristics of transdermal matrices and the ability to deliver both hydrophilic and hydrophobic drugs and beneficial phytochemicals. Sodium alginate is one of the most abundant, easily-available, economic, biocompatible, stimuli-responsive, and smart hydrogel forming natural polysaccharides and has been considered as a potential

polymer for the fabrication of effective TDDSs.^{12,13} In this chapter, different sources, chemistry, physicochemical, and biological properties of alginate, various alginate-based TDDSs along with their preparation methods, and various applications have been discussed.

3.2 Alginate: Sources, Physicochemical, and Biological Properties

3.2.1 Sources of Alginates

Alginates are obtained from three marine brown algae *Laminaria hyperborea*, *Ascophyllum nodosum*, and *Macrocystis pyrifera* belonging to the family Phaeophyceae and some bacteria such as *Azotobacter vinelandii* and pseudomonas species.^{12,14} Alginates from algae sources have zero degree of acetylation, whereas bacterial alginates have a significantly high degree of acetylation and are usually not used for commercial purposes.¹⁵ The monosaccharide ratio (mannuronic acid/guluronic acid) and degree of acetylation vary with variation in bacterial species and their growth conditions. The degree of acetylation affects the flexibility and viscosity of alginate. The monosaccharide contents, their sequences, molecular weights, and compositions of alginates significantly vary with the sources and species. Alginates usually exist inside the cells of the brown marine algae as a combined salt of seawater cations such as Na^+ , Ba^{2+} , Mg^{2+} , and Sr^{2+} , etc.^{16,17}

3.2.2 Physicochemical Properties

Alginate is an anionic hydrophilic unbranched linear polysaccharide chemically composed of the irregular blocks of 1 → 4 linked β-d-mannuronic acid (M-block) and 1 → 4 linked α-l-guluronic residues (G-block) (Figure 3.1).^{18–21} The organization of the block-structure of alginate may be homogeneous or heterogeneous.¹⁴ The homopolymeric blocks are generally of either repeated mannuronic acid residues (MMMMMM) or repeated guluronic acid residues (GGGGGG), where the heteropolymeric blocks comprise alternating M and G residues (GMGMGM).²² The

monosaccharide contents, their ratio, distribution pattern of M-blocks and G-blocks, linkage between the monosaccharide, and geometries of the blocks greatly influence the physicochemical properties of alginates such as their viscosity, gelation, and water absorption. Alginic acid is insoluble in water, whereas salts of alginate with monovalent cations are water soluble. Alginates with poly-mannuronic acid or poly-guluronic acid structures precipitate at low pH, while those with alternative MG-blocks are found to be soluble under the same conditions. The valence of gelling cations and ionic strength also influence the solubility of the alginate salts.

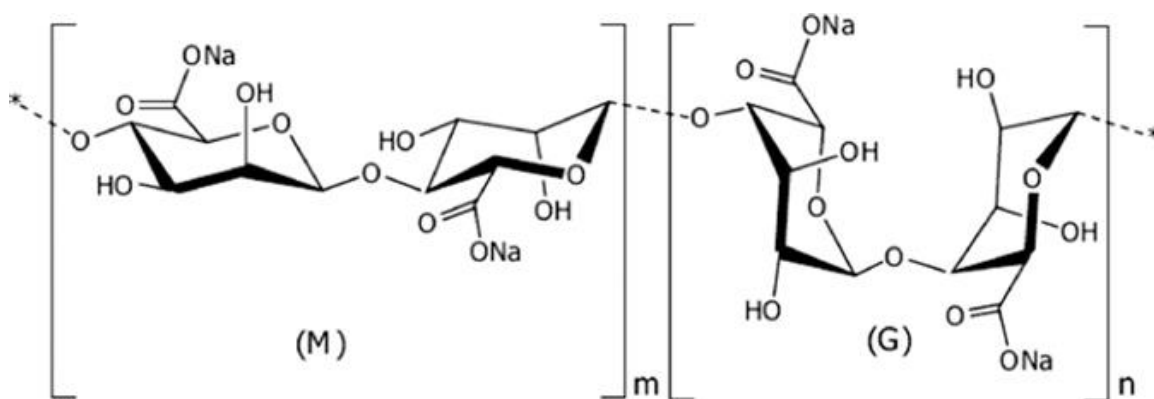


Figure 3.1 Chemical structure of sodium alginate: (M) block for β-d-mannuronic acid, and (G) block for α-l-guluronic acid.²¹ Reproduced from ref. 21 with permission from Elsevier, Copyright 2011.

The viscosity of the alginate solution is found to be dependent on the pH of the solution. A decrease in the pH value results in an increase in the viscosity due to the protonation of the carboxylate ions in the alginate-backbone and subsequent formation of hydrogen bonds. The average molecular weight of alginate varies from 33 000 to 400 000 g mol⁻¹. An increase in the molecular weight of the alginate results in a more viscous and stiff gel compared to that of lower molecular weight. Alginate has been used in the food and pharmaceutical industries as a suspending agent, emulsifier, and viscosity-building agent due to its excellent gelling capacity.²³

Intramolecular and intermolecular cross-linking of alginate molecules occur in the presence of different divalent or trivalent metal cations (except

Mg²⁺), which results in the formation of ionotropically-gelled matrices.^{24–26} When an aqueous dispersion of alginate is introduced into solutions containing multivalent metal cations, the cations are covalently bonded to a number of –COO[–] ions of alginate molecules depending upon the valence of the cations leading to the formation of ionotropically cross-linked networks (*i.e.*, “egg-box” model).^{27–29} This is a temperature-independent process. The α-l-guluronate units of G-blocks in the polysaccharide-chains could distinctly amplify their selective binding to the polyvalent metal ions, resulting in tightly held junctions. Similarly, the MG-blocks of alginate also contribute to this process, but result in a relatively weaker junction.²³ Accordingly, the alginates with higher guluronic acid content could achieve more rigid hydrogels.³⁰ Better lateral association of G-blocks of alginate molecules with the rising cations beyond their dimerization has been found in X-ray scattering analysis of alginate gels.³¹ The ionotropically cross-linked alginate gels are less stable in the presence of different non-crosslinking ions such as Na⁺, K⁺, and Mg⁺⁺. An augmentation in the guluronic acid content of the alginate molecules could enhance the stability of the ion-cross-linked gel-matrix.

3.2.3 Biological Properties of Alginates

3.2.3.1 Immunogenicity

There are various commercial grades of alginate available on the market including food grade, research grade, and ultra-pure grade. Research-grade alginate has been found to contain some mutagenic impurities which can promote the release of cytokine and inflammatory reactions when administered.¹⁴ It has also been reported that the alginate promotes the production of cytokine depending on its mannuronic acid content.³² On the other hand, the ultra-pure grade of alginate shows evidence of a minimal immunogenic response because of its high α-l-guluronic acid and low β-d-mannuronic acid contents.^{33,34} The alginate rich in mannuronic acid content stimulates the production of inflammatory protein cytokine and thereby promotes an immunological response through signaling to toll-like receptors such as TLR-2 and TLR-4 as well as CD 14.³⁵

3.2.3.2 *Bio-Adhesive Property of Alginates*

Alginates have numerous carboxyl groups in their polysaccharide-backbone, which impart a strong bio-adhesive capacity to alginates and make them suitable for various mucoadhesive and transdermal drug delivery systems. Alginate often exhibits greater bioadhesion compared to some other biopolymers such as chitosan, poly (lactic acid), polystyrene, and carboxymethylcellulose, *etc.* The promising bio-adhesive potential of the alginate could be utilized in the fabrication of drug delivery devices with different intentions such as site-specific drug-delivery and bioavailability enhancement.¹⁴

3.3 Preparations of Different Alginate-based Transdermal Systems

3.3.1 Alginate Films

Sikareepaisan and co-researchers have described an alginate-based film loaded with asiaticoside for the development of efficient wound-healing systems. At first, a base alginate solution with a fixed concentration of 2% w/v was prepared. A 0.05% w/v aqueous solution of calcium chloride was slowly added to the base alginate solution with constant stirring for 4 hours. Separately, asiaticoside powder was dispersed in water and sodium alginate was then dissolved in the drug suspension followed by the addition of a 0.05% w/v aqueous solution of calcium chloride. Finally, the alginate solution and asiaticoside suspension were placed into 12.5 cm × 22.5 cm polystyrene plates and subsequently dried at 50 °C for 4 days. The films thus obtained were designated as “mixed” films which were further cross-linked by placing in a calcium chloride aqueous solution only for a 10 s period, dried at 50 °C in a hot air oven, and designated as “immersed” films.²¹

Another alginate-film loaded with a polyvinylpyrrolidone–iodine complex (PVPI) was demonstrated by Liakos *et al.*³⁶ The films were cast from three identical sodium alginate aqueous stock solutions with various concentrations of PVPI. The alginate solution was preheated at nearly 100

°C in a hot plate for 1 h to ensure complete dissolution and then allowed to cool to ambient temperature. A constant amount of glycerol was added to each alginate solution as a plasticizer. A specified volume of aqueous PVPI solution was mixed with a previous sodium alginate–glycerol mixture while vortexing and left for some time to allow the evolution of bubbles if present. Finally, 200 µl of the resultant mixture was dropped on glass slides of 1 cm² area and left in ambient laboratory conditions for air-drying.

A lornoxicam-loaded matrix-type film of sodium alginate was reported by Shivakumar and co-researchers.³⁷ The films were prepared using a solvent-casting method. For the preparation of the film, lornoxicam was first dissolved in a minimum quantity of ethanol and diluted to 15 ml with phosphate buffer with a pH of 7.4. Sodium alginate was then added and dissolved in the solution of the drug and the resultant dispersion was cast in a Petri-plate of 4.5 cm in diameter. Glycerin was also added to the mixture as a plasticizer before casting. A circular film with 1 cm diameter was cut out after drying at room temperature for 48 h. Different formulations were prepared with different permeation enhancers. The films were further treated with calcium chloride solution (10% w/v) to increase the hardness of the films. A similar method was also adopted to prepare another transdermal film, which utilized the combination of two natural polysaccharides, sodium alginate and xanthan gum for transdermal delivery of domperidone. The drug was dissolved in the solution of sodium alginate and xanthan gum and 2%w/w glycerin was added to the drug–polymer mixture as a plasticizer. Finally, the resultant dispersion was poured onto a petri dish and dried under vacuum to obtain a drug-loaded polymeric membrane.³⁸ A methotrexate-loaded transdermal membrane composed of sodium alginate and polyvinylpyrrolidone was described.⁵

3.3.2 Alginate-based Microneedles for Transdermal Drug Delivery

The microneedle (MNs) based transdermal drug delivery systems describe the use of small needle-shaped structures to form micron-sized pores on the surface of the epidermis to promote the permeation and delivery of drugs to the systemic circulation. MNs diverge from a conventional transdermal film/membrane since they act not only at the place of application (stratum

corneum) but also deliver drugs to deeper layers of the skin such as the dermis and hypodermis as well as to the blood vessels of skin. MNs do not penetrate to the deeper layers of the skin and therefore do not cause pain and bleeding.³ Although there have been various reported methods for the preparation of microneedles, micromoulding has been the most widely used method. Firstly, a negative mould usually made of polydimethylsiloxane is produced using a positive master mould made of a strong material such as metal or silicon. Polydimethylsiloxane is generally selected for the preparation of negative mould due to its thermostability, flexibility, and reproducibility.³⁹ The negative moulds are used to produce the microneedle-arrays.⁴⁰ This technique has been frequently used to produce polymeric microneedles composed of particularly hydrogel-forming polysaccharides such as alginate, chitosan, *etc.* Micromoulding has been considered as an easy approach to use and is cost-effective. It allows the incorporation of less stable drugs due to its mild preparation. But at the same time some problems are also associated with this approach such as inadequate wetting of the polydimethylsiloxane mould due to high interfacial tension, the advance cooling due to the higher viscosity of the polymers, and development of mould imperfections. However, various strategies such as centrifugation or a vacuum-filling step have been adopted to overcome the limitations.⁴¹ Moreover, a wide variety of negative moulds composed of polydimethylsiloxane with a variety of designs are commercially available with variation in size, shape, and number of tips.¹

3.3.3 Alginate-based Electroresponsive Transdermal Drug Delivery System

An alginate-based electroresponsive TDDS (ETDDS) was described by Kulkarni and co-researchers, where alginate was first grafted with polyacrylamide by a free-radical initiation method using ammonium persulfate as the redox-initiator in a nitrogen environment and the graft-copolymer was then partially hydrolyzed by sodium hydroxide in order to convert some $-\text{CONH}_2$ groups of acrylamide into $-\text{COOH}$ groups. The system was prepared by entrapment of the hydrolyzed copolymer in a reservoir in a thin compartment mould. The mould had two sides – one was

an impermeable backing layer and another was a rate-controlling membrane as shown in Figure 3.2.^{42,43}

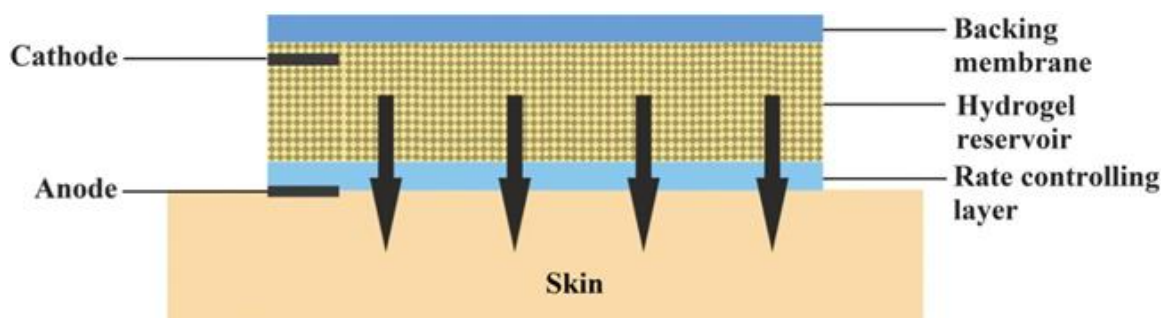


Figure 3.2 Schematic representation of electro-responsive TDDS.

The alginate copolymer was first dissolved in water for the preparation of a hydrogel reservoir. The drug ketoprofen was then uniformly dispersed in the previous solution of alginate copolymer followed by the addition of glutaraldehyde and 1% 1 N HCl acid with constant stirring for 4 h. Finally, the resultant cross-linked hydrogel was washed with water and preserved in a closed container. The rate-controlling membrane was prepared with polyvinyl alcohol (PVA) and poly(ethylene glycol)-200 (PEG) as a plasticizer. The mixture of PVA and PEG was poured on a mercury surface of 28.3 cm² in a petri dish and dried at ambient laboratory conditions for 24 h. The resultant membrane was also cross-linked with glutaraldehyde, washed with water, and air-dried. Polystyrene film laminate was used as the backing membrane. The hydrogel-reservoir was placed in between the backing membrane and rate-controlling membrane and the edges were heat-sealed.

3.4 Drug Delivery Application of Alginate Based Transdermal Carriers

Several alginate-based transdermally applied drug delivery systems are summarized in Table 3.1.

Table 3.1 Alginate-based transdermal drug carrier for therapeutic applications.

S. no	Application	Formulation	Drug	Ref.
1	Antidiabetic	Microneedle array patch	Insulin	44
		Microneedle array patch	Insulin	45
		Microneedle array patch	Insulin	46
		Microneedle array patch	Glucose oxidase and exendin-4	47
2	Anticancer	Nano-rod incorporated patch	Methotrexate	48
		Transdermal membrane	Methotrexate	5
		Nanoparticle incorporated patch	Curcumin and 5-flurouracil	49
3	Anti-inflammatory	Transdermal gel	Ibuprofen lysinate	50
		Transdermal patch	Paracetamol	51
		Mucoadhesive hydrogel	Ketorolac tromethamine	52
		Nanoparticles	Glucosamine sulfate	53
4	Antibiotics	Microparticles incorporated thermogel	Doxycycline	54
		Transdermal patch	Ciprofloxacin	55
		Nanofibrous membranes	Moxifloxacin hydrochloride	56
		Transdermal dressing	Ciprofloxacin	57
		Nanofiber patches	Gatifloxacin hydrochloride	58
		Halloysite nanotubes incorporated in gel formulation	Vancomycin	59
		Transdermal film	Metronidazole	60

5	Antihypertensive	Matrix type transdermal patch	Diltiazem hydrochloride	61
		Hydrogel transdermal membrane	Prazosin hydrochloride	62
6	Antifungal	Nanocapsules	Ebselen	63
		Bioadhesive gel	Fluconazole	64
		Bioadhesive gel	Propolis	65
7	Antimicrobial	Transdermal film	Curcumin & cutaneous antiseptic	66
		Transdermal patch	Trolox [®]	67
		Transdermal hydrogel	Nano-silver	68
		Transdermal dressing	Silver nanoparticles	69
		Transdermal fibrous mats	Zinc oxide	70
8	Antioxidant	Nanocarrier incorporated membrane	Curcumin	71
		Hydrogel nanocarriers	Curcumin	72
		Transdermal patch	Epicatechin	73
9	Anti-alopecia	Transdermal hydrogel	Minoxidil	74
		Transdermal hydrogel	Minoxidil	75
		Aqueous transdermal gel	Minoxidil	76
10	Miscellaneous	Microneedle based patch	BCG vaccine	77
		Matrix type transdermal patch	Donepezil	78
		Transdermal films	Ketotifen fumarate	79
		Nanoparticle incorporated transdermal patches	Rabeprazole	80

3.4.1 Delivery of Antidiabetic Agents

Wu *et al.* have fabricated a microneedle based patch system causing minimal invasiveness when delivering insulin. 3D printing technology was used to develop glucose responsive microneedles as shown. The printability of alginate-based bioinks was investigated. The base diameter, tip diameter, and height of the conical microneedles were found to be 601 μm , 24 μm , and 643 μm respectively. A study conducted in diabetic mice showed a glucose responsive release of insulin regulating the glucose level for up to 40 h.⁴⁴

The efficacy of polymeric microneedles based on hyaluronate and alginate were investigated by Yu *et al.* for transdermal application of insulin. The developed microneedles showed exceptional mechanical strength for skin permeation. A microscopic study demonstrated gradual diffusion of the drug into the deeper tissue layers. The pharmacodynamic tests conducted on diabetic mice demonstrated good feasibility of administration. A relative bioavailability of $92.9 \pm 7\%$ and relative pharmacological activity of $90.5 \pm 6.8\%$ indicated the efficacy of alginate based microneedles for delivering antidiabetic agents.⁴⁵

Alginate and maltose based microneedles were fabricated for transdermal insulin delivery by Zhang *et al.* using an ionic cross-linking technique. The microneedles exhibited a failure force as high as 0.41 N per needle indicating a good mechanical strength. An *in vivo* study was conducted on diabetic Sprague-Dawley rats and the study reports showed an effective hypoglycemic efficacy with a relative pharmacological activity of $94.1 \pm 5.6\%$. The relative bioavailability of insulin was reported as $93.7 \pm 4.7\%$ with respect to traditional subcutaneous insulin administration.⁴⁶

Chen *et al.* synthesized other alginate-based microneedle array patches for delivering glucose oxidase and exendin-4. The developed patches showed a glucose responsive selective release of exendin-4 in a closed loop manner enhancing the therapeutic efficacy of exendin-4. The dual integrated mineralized particles were also reported to increase the mechanical strength of the microneedle system. The synthesized patches showed great potential for transdermal delivery of exendin-4 as a non-invasive, long term treatment option for type 2 diabetes.⁴⁷

3.4.2 Delivery of Anticancer Agents

The efficacy of alginate-based transdermal patches in cancer theranostics was investigated by Matai *et al.* Alginate in combination with polyacrylamide was employed to fabricate an interpenetrating polymeric network encapsulating an anticancer drug, methotrexate. Gold nano-rods and polyvinylpyrrolidone grafted graphene oxide were used to develop the hybrid nanostructured inner part of the patch. Scanning electron microscopic images exhibited a porous surface for the synthesized hybrid hydrogel. The developed patch demonstrated photo-thermal behavior after exposure to near-infrared radiation, indicating a rise in surface temperature which may cause thermal removal of cancer cells. The photo-thermal stability was reported to be retained for up to four cycles. The release of methotrexate was found to be triggered by near-infrared radiation.⁴⁸

Another study involving methotrexate was conducted by Bajas *et al.* who developed alginate-based transdermal membranes for topical application of chemotherapeutic agents. The efficacy of various copolymers, polyvinylpyrrolidone, tween, and carbopol was investigated in combination with sodium alginate. The binary mixtures developed during the membrane synthesis were characterized by thermogravimetric methods. Carbopol and alginate-based membranes exhibited non-homogenous membrane formation.⁵

Two commonly used anti-cancer agents – curcumin and 5-fluorouracil were incorporated into a transdermally applied drug delivery system. Alginate-modified aminated nanodextran and chitosan-modified folate linked aminated β -cyclodextrin nanocarriers were used for synthesizing the transdermal carrier. Surface charge induced electrostatic interaction caused coupling of the polymers. The fabricated system was evaluated for solvent selectivity which demonstrated curcumin and 5-fluorouracil release with 1-butanol and ethanol respectively in a sustained manner. The *in vitro* drug release study was performed in a Franz diffusion cell using rat skin. The skin irritation study and adhesion test showed good results. The study results indicated that the use of selective solvents enhanced the drug permeation across skin. Histological and biological investigation performed on cancer cells and skin showed the efficacy of the combinatorial chemotherapeutic agents in skin carcinoma.⁴⁹

3.4.3 Delivery of Anti-inflammatory Agents

The efficacy of modified alginate was investigated for topical administration of ibuprofen lysinate by Al-Remawi *et al.* Oleoyl alginate was developed and optimized based on surface tension, viscosity, and degree of substitution. The synthesized oleoyl alginate was characterized and further employed in gel formation. A skin permeation study for the prepared polysaccharide gel was performed using a Franz diffusion cell which exhibited ibuprofen lysinate release over 6 h through rabbit skin. Depending on the surface tension, the highest particle size was observed for the optimized formulation of oleoyl alginate.⁵⁰

In order to avoid the gastrointestinal complications caused by oral delivery of paracetamol, Sami *et al.* developed a transdermally deliverable system based on biopolymers. The transdermal patches consisting of sodium alginate, guar gum, and chitosan were evaluated for their physicochemical behavior. The cytotoxic profile and biocompatible nature were evaluated using HeLa cell lines. The developed hydrogel was used to prepare paracetamol encapsulated alginate beads in a transdermal matrix. Avian skin was used to study the drug release pattern from the transdermal patch which indicated zero order drug release following a non-Fickian mechanism. The alginate-based hydrogel patch showed great potential for sustained paracetamol delivery through a transdermal route.⁵¹

A study involving ketorolac tromethamine for managing pain and inflammation occurring during anogenital condylomas removal was carried out by El Moussaoui *et al.* An alginate-based hydrogel carrier was developed for minimizing the inflammation and pain after anogenital wart surgery. The hydrogel exhibited the required physical and mechanical behavior intended for topical application. A one phase exponential model can be followed for ketorolac tromethamine release which may retain on vaginal mucosal layer. An *in vivo* study on humans showed good tolerability and absence of visible irritation. The anti-inflammatory study report indicated 53% reduction in inflammation *in vivo*.⁵²

The efficacy of alginate nanoparticles as a transdermal carrier was investigated for delivering glucosamine sulfate for osteoarthritis pain management. The developed nanocarriers were evaluated for surface charge, zeta potential entrapment efficiency and yield. *In vitro* glucosamine

sulfate release was reported to be in a sustained manner following a Higuchi model. An *ex vivo* permeation study performed using rat skin indicated $86.94 \pm 5.28\%$ of glucosamine sulfate released over 7 h. The negative zeta potential (-47.1 mV) of the alginate based nanoparticles may enhance the drug permeation through human skin ($+23$ mV).⁵³

3.4.4 Delivery of Antibiotics

A hydrogel-based microparticulate delivery system was developed by Giovagnoli *et al.* for intradermal administration of doxycycline. Alginate-doxycycline microparticles were synthesized using a calcium chloride solution with an ionic gelation technique. The microparticles were embedded into a thermogel of pluronic F127 for facilitating transdermal application. 90% of doxycycline release was reported within two days. A much faster drug release of 80–90% was observed within 4 h in the presence of methocel. A thermogel containing pluronic F127-hydroxypropyl methylcellulose exhibited a linear doxycycline release in a sustained manner for 7 days.⁵⁴

Ciprofloxacin-loaded sodium alginate and a poly vinyl alcohol-based nanofiber containing transdermal patches were developed for infection control by Kataria *et al.* An active loading process was followed for drug loading into electrospun nanofibers. The drug encapsulation was confirmed by a swelling study and scanning electron microscopic technique. The drug release from the nanofiber-based transdermal system followed Korsmeyer-Peppas and Higuchi models. *In vivo* studies performed on a rabbit model showed a rapid wound healing ability with respect to an unloaded patch indicating the presence of higher hydroxyproline in the wound bed.⁵⁵

Another study based on sodium alginate and poly vinyl alcohol was reported by Fu *et al.* for transdermal delivery of moxifloxacin hydrochloride. Nanofibrous membranes were developed using sodium alginate as a drug carrier and polyvinyl alcohol as a permeation enhancer. The drug release characteristics from electrospun nano-fibermats were investigated which demonstrated 80% of moxifloxacin hydrochloride release after 10 h. Drug loaded nano-fibermats showed accelerated wound healing along with antibacterial efficacy.⁵⁶

Ahmed *et al.* investigated the effectiveness of calcium alginate-based wafer dressings for transdermal delivery of ciprofloxacin to treat diabetic foot ulcers. X-ray diffraction analysis indicated the crystalline structure of the dressing. The drug release study reflected an initial rapid release which eventually slowed down in a sustained manner preventing the phenomenon of re-infection. The cellular viability was reported to be >85% after 72 h indicating good biocompatibility with human keratinocytes.⁵⁷

Another type of novel electrospun nanofibers was developed by Arthanari *et al.* for topical administration of gatifloxacin hydrochloride. Alginate and polyvinyl alcohol-based biomedicated nanofiber patches were developed to achieve rapid wound healing. The encapsulation of gatifloxacin hydrochloride in nanofibers was evidenced by the results obtained from X-ray diffraction, scanning electron microscopy, swelling characteristics, and differential scanning calorimetry. The drug release study from the patch was performed using 7.4 pH phosphate dissolution media which showed sustained release of gatifloxacin hydrochloride for up to 24 h. The study results suggested that the initial drug release pattern from a nanofiber-based patch has influenced the nanofiber thickness.⁵⁸

An antibiotic entrapped alginate-based transdermal dressing was developed by Kurczewska *et al.* The effect of halloysite nanotubes on vancomycin release was studied when using alginate or alginate/gelatin gels. The study finding suggested that the presence of gelatin helps to prolong the release of vancomycin. The drug release followed the Higuchi model and maintained Fickian diffusion. The antimicrobial analysis demonstrated a greater inhibition zone than the standard disks for the enterococci and staphylococci bacteria tested. The toxicity study performed using an *in vivo* model of *Daphnia magna* and *Acutodesmus acuminatus* showed no signs of toxicity.⁵⁹

Sodium alginate-based transdermal films were developed for wound dressings by Sarheed *et al.* A freeze–thaw technique was employed along with calcium ion-induced ionotropic gelation to fabricate the metronidazole-loaded hydrogel films. The drug release from the hydrogel matrix followed Fickian diffusion. The *in vivo* wound healing study showed a significant ($p < 0.05$) decrease in wound size after 5 days and 10 days of treatment.⁶⁰

3.4.5 Delivery of Antihypertensive Agents

Anirudhan and his coworkers have developed a transdermal matrix based on sodium alginate, polyvinyl alcohol, and carboxymethyl cellulose for delivering the widely used antihypertensive drug diltiazem hydrochloride. The drug loading study indicated the good drug loading ability for all the developed patches. The *in vitro* permeation study showed efficacy of the transdermal matrix indicating a cell viability of more than 40% on PBMC and HaCaT cell lines with negligible histological changes.⁶¹

Kulkarni *et al.* developed sodium alginate and poly(vinyl alcohol)-based hydrogel membranes through a solvent casting technique. An antihypertensive drug prazosin hydrochloride was incorporated in the interpenetrating polymer network with the help of a cross linking agent glutaraldehyde. An amorphous dispersion of prazosin hydrochloride was confirmed by X-ray diffraction study. The *in vitro* drug permeation study conducted on excised abdominal skin of rats demonstrated drug release depending on glutaraldehyde concentration. A sustained release of prazosin hydrochloride up to 24 h was reported. A skin irritation study indicated that the developed polymeric interpenetrating network system showed relatively less irritability.⁶²

3.4.6 Delivery of Antifungal Agents

Alginate-based nanocapsules were developed by Jaromin *et al.* for intradermal delivery of ebselen. The particle size of the developed nanocapsules was found to be less than 204 nm. A negative zeta potential of -17.1 with a satisfactory polydispersity index was reported. The ebselen loaded nanocapsules showed marginal toxicity toward dermal fibroblasts in human. The *ex vivo* permeation study performed on pig ear skins demonstrated little transdermal passage and good interaction was confirmed by differential scanning calorimetry with stratum corneum. The antifungal efficacy was tested against *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata* strains which exhibited promising results in the treatment of candidiasis.⁶³

Bioadhesive gels containing sodium alginate, hydroxylpropyl methylcellulose, and poly(acrylic acid) were formulated by Gafitanu *et al.* for vaginal delivery of fluconazole. An inclusion complex of fluconazole- β -

cyclodextrin was prepared and incorporated in the vaginal gel in 1% wt/wt. An improved delivery of fluconazole was reported following quasi-Fickian, Fickian, and non-Fickian diffusion. The highest drug release of 92% was reported after 43 h. The fluconazole- β -cyclodextrin inclusion complex showed selective efficacy against *Candida* strains and *S. aureus* with respect to the hydrogel only with fluconazole.⁶⁴

Andresa *et al.* investigated the efficacy of a traditional bee product, propolis in the treatment of vaginal infection caused by *Candida albicans*. The antifungal behavior of the propolis was studied through vaginal gel formation using sodium alginate, carbopol, pectin, poloxamer, and chitosan. The chemical analysis along with mucoadhesive and rheological behavior were also evaluated. Good fungicide activity was recorded against yeast, hyphae, and pseudohyphae. The maximum antifungal activity was exhibited after 6–8 h of incubation. All the developed gels showed low thixotrophy. The *in vivo* study indicated an antifungal efficacy equivalent to clotrimazole cream.⁶⁵

3.4.7 Delivery of Antimicrobial Agents

The efficacy of polymeric film containing sodium alginate and MaterBi[®] was investigated by Setti *et al.* for delivering hydrophobic and hydrophilic drugs. A hydrophilic cutaneous antiseptic agent and curcumin were selected as model drugs to be incorporated in the developed emulsion. The aqueous phase was made of sodium alginate and the oil phase contained hydrophobic MaterBi[®]. Emulsification was achieved using an ultrasonic method without adding surfactants. Physically stable emulsions were formed which were dried depending on the polymeric ratio. The drug release ability was investigated and controlled drug release was achieved depending on the polymer fraction or sodium alginate crosslinking with calcium ions. The developed films exhibited excellent biocompatibility with human fibroblasts.⁶⁶

Nayak *et al.* developed silver nanoparticle-coated dermal patches for preventing microbial infections. The fabricated biopolymer based patch contains keratin, alginate, agar, and gellan gum. Glucose oxidase was incorporated for controlling the glycemic level. An antioxidant agent Trolox[®] was incorporated for improved wound healing. The mechanical

strength was found to be excellent. 77.77 to 88.89% release of fibroblast growth factor was recorded from the patches after 3 days. All the developed patches using various polymeric combinations were found to be efficient against various microorganisms as shown in Figure 3.3.⁶⁷

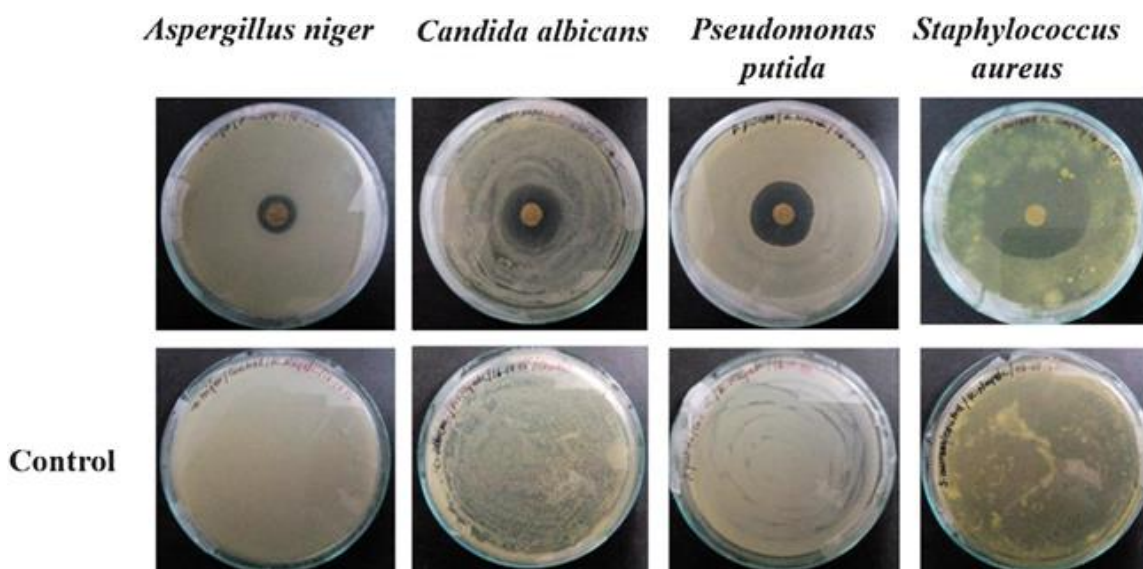


Figure 3.3 Antimicrobial efficacy of alginate-based transdermal patches against various microorganisms.⁶⁷ Reproduced from ref. ⁶⁷ with permission from Elsevier, Copyright 2017.

The antimicrobial efficacy of nano-silver was investigated by employing dehydrated alginate and its ion-exchange response. The nano-silver alginate model was fabricated using hydrogel formation followed by dehydration. The developed composite showed excellent biocompatibility. The release of nano-silver was controlled by the self-regulated swelling behavior of the composite. The nano-silver is reported to maintain a constant range of concentration required for producing antimicrobial efficacy without exhibiting cytotoxicity. A study conducted with a murine model showed significant reduction in bacteria count indicates the efficacy to deliver antimicrobial agents in dermal wounds.⁶⁸

Chabala *et al.* developed silver nanoparticle-incorporated alginate–chitosan films as a novel wound dressing material. *Aloe vera* gel was used for imparting emollient properties to the film. In the polymeric blend of alginate, chitosan, and aloe vera gel, silver nanoparticles were incorporated

using an immersion technique. The antibacterial property was characterized by using *P. aeruginosa* and *S. Aureus*. The results of the study suggested that the combination of emollient and antibacterial properties makes it favorable for minor wound dressing.⁶⁹

Shalumon *et al.* used an electrospinning technique to formulate sodium alginate–poly (vinyl alcohol)-based fibrous mats for delivering zinc oxide nanoparticles. The synthesized fibrous nanocomposites were evaluated by thermogravimetric analysis and scanning electron microscopy. The cytocompatibility study indicated that at 0.5 to 1% zinc oxide concentration, the toxicity produced is much lower than with an increased zinc oxide concentration. *E. coli* and *S. aureus* have been used to evaluate the antibacterial efficacy of zinc oxide which demonstrated good antibacterial activity.⁷⁰

3.4.8 Delivery of Antioxidants

The antioxidant activity of a hydrophobic drug, curcumin, was investigated in combination with alginate-based novel nanocarriers. Calcium alginate-based nanocarriers were synthesized by polymeric crosslinking through a nanoemulsification technique. The nanocarriers contain a hydrophobic oily core surrounded by a polymeric membrane of alginate. The hydrodynamic diameters of the developed nanocarriers were reported as 200 nm with a zeta potential of –30 mV. The encapsulation efficiency of curcumin was 95% retaining a complete antioxidant property. A study performed on human skin exhibited accumulation of curcumin above skin layers indicating the potential of calcium alginate nanocarriers for topical antioxidant delivery.⁷¹

Miloudi *et al.* developed curcumin loaded nanocarriers and investigated the feasibility for delivering the drug through the skin. The alginate-based hydrogel nanocarriers were analyzed using Raman spectroscopy and infrared spectroscopy which showed direct tracking of the alginate nanocarriers.⁷²

Alginate-based transdermal patches for delivering antioxidant agents were developed by Silva *et al.* Several cross linkers were used to evaluate their effect on the morphological characteristics and shrinkage behavior. The release behavior of the encapsulated flavonoid, epicatechin, was

reported to vary based on the thickness of the film and the metallic cross linker used. An *ex vivo* study exhibited the ability of the alginate-based patch to deliver the drug in the required concentration. The released epicatechin showed full antioxidant efficacy indicating the efficacy of the developed patch.⁷³

3.4.9 Delivery of Anti-alopecia Agents

The efficacy of an alginate-based carrier for delivering an anti-alopecia drug, minoxidil, was investigated using topically applied hydrogel formulations. Various hydrogel forming polymers were used including alginates, carbopol 934, and hydroxyethyl cellulose to develop an aqueous hydrogel for transdermal application. A freeze-drying technique was employed to develop an inclusion complex with methyl- β -cyclodextrin to improve the solubility of minoxidil. The formulated polymeric hydrogels were evaluated for their rheological properties, drug release behavior, and *ex vivo* skin permeation study. The study findings suggested the calcium alginate-based gel formulation as the best carrier system for transdermal delivery of minoxidil.⁷⁴

In another study, Lopedota *et al.* incorporated non-aqueous solvents to study the efficacy of an alginate-based carrier for delivering an anti-alopecia drug, minoxidil. Ethanol and propylene glycol were employed as co-solvents for developing an alginate-based hydrogel formulation. An inclusion complex of minoxidil was formed using hydroxypropyl- β -cyclodextrin by varying the degree of molar substitution. A 22-fold aqueous solubility enhancement was reported for minoxidil at 39% w/v concentration. Rheological evaluation indicated an increased viscosity of hydrogel upon the formation of an inclusion complex. The *in vitro* drug release suggested minoxidil permeation through the skin without damaging the epidermis layer.⁷⁵

Another study performed by Tricarico *et al.* involved an inclusion complex between minoxidil and hydroxypropyl- β -cyclodextrin incorporated into an alginate-based gel formulation. The developed transdermal formulations were characterized for hair growth ability using depilated dorsal skin which demonstrated a relatively higher efficacy of the inclusion complex minoxidil than free minoxidil and the control group as shown in

Figure 3.4. The inclusion complex of minoxidil was found to increase the mRNA levels of Kir6.1 and SUR2 subunits indicating favorable long term topical use of alginate-based gels.⁷⁶

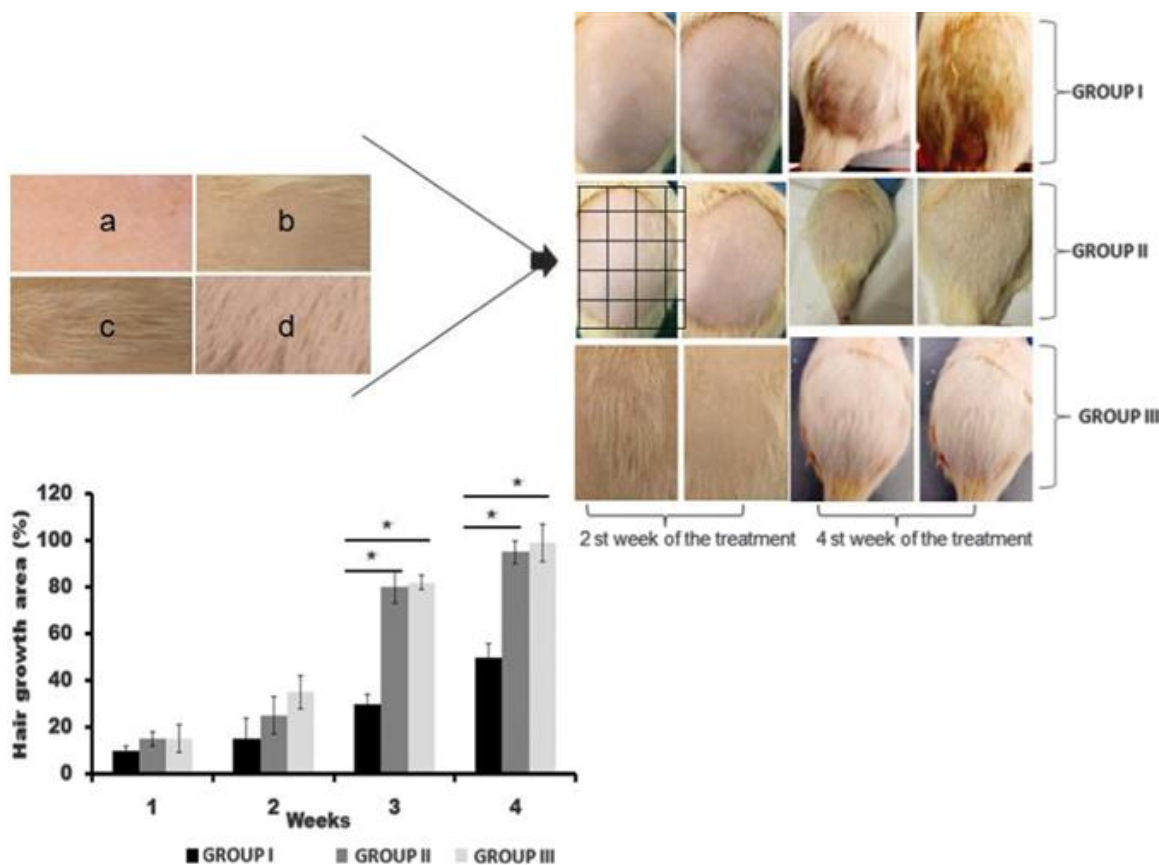


Figure 3.4 Images of dorsal skin after topical application of minoxidil incorporated alginate-based gel formulation.⁷⁶ Reproduced from ref. 76 with permission from Elsevier, Copyright 2018.

3.4.10 Miscellaneous

Microneedle patches were developed using sodium alginate and investigated for transdermal delivery of the BCG (Bacillus Calmette-Guérin) vaccine. The developed patches showed 0.8 mm of thickness and a folding endurance greater than 300. The IgG ELISA assay and hematological studies indicated lymphocyte and granulocytes production along with an activated immune response. The increased number of

leukocytes and IgG antibodies showed the efficacy of the transdermal vaccine delivery system through alginate microneedles.⁷⁷

Galipoğlu *et al.* developed a matrix type transdermal patch for delivering donepezil in the therapy of Alzheimer disease. dl-Limonene and propylene glycol were employed as a penetration enhancer and plasticizer. The study findings indicated suitable bioadhesive and mechanical properties for transdermal application. The skin permeation of donepezil exhibited higher drug permeation with 3% dl-limonene.⁷⁸

An anti-asthmatic drug, ketotifen fumarate was incorporated in transdermal delivery containing sodium alginate and chitosan. Polyelectrolyte complex-based transdermal films were developed using a film casting technique. The developed formulations were reported to have suitable physicochemical properties for transdermal administration. The drug release was found to be over 99% after 24 h following the Korsmeyer–Peppas mechanism.⁷⁹

Ahmed *et al.* investigated the efficacy of nanoparticle incorporated transdermal patches for antacid delivery. A nanoemulsification technique was employed to develop rabeprazole-loaded alginate–chitosan nanoparticles which were further formulated into a transdermal matrix to avoid gastric acid sensitivity. The *ex vivo* skin permeability was performed using excised rat skin which exhibited excellent permeability with controlled release of rabeprazole maintaining Fickian diffusion.⁸⁰

3.5 Conclusion

Alginate is an anionic polysaccharide widely used in the fields of drug delivery, cosmetics, and the food industry. The attractive physicochemical properties of alginate have attracted the attention of pharmaceutical scientists. The rheological behavior of alginate combined with the ionic crosslinking and bioadhesive properties has added certain superiority to this natural polysaccharide. The versatility of alginate has encouraged researchers to investigate its efficiency in transdermal delivery of therapeutics. Various delivery techniques have been employed including microneedle patches, transdermal matrix patches, transdermal films, and topical gel formulation. The intradermal delivery of several therapeutic

agents has been highlighted in this chapter reflecting the adaptability, flexibility, and efficacious nature of alginate applied in transdermal delivery of drugs.

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Chapter 4

Chitosan-based Nanocarriers for Drug Delivery: Advances and Challenges

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4.1 Introduction

In recent decades, the design of nanocarriers for drug delivery has emerged as a promising strategy to improve the therapeutic efficacy of pharmacologically active agents.^{1,2} Such drug delivery systems (DDS) have been designed using a broad range of materials and chemical strategies exhibiting numerous advantages including enhanced drug encapsulation, controlled release of active molecules, prolonged circulation time, improved targeting capability, enhanced cellular uptake, stability *in vivo*, as

well as the potential to reduce side effects.³ DDS engineering is challenging and requires carriers that can load a sufficient quantity of active pharmaceutical ingredients (API), stabilize the API *in vivo*, and control their release for appropriate periods of time at targeted locations within the body.¹

A variety of materials, such as natural⁴ or synthetic⁵ polymers, lipids,⁶ and proteins⁷ have been employed in the design of drug nanocarriers. Among them, polysaccharides have gained considerable interest in recent years due to their outstanding physical and biological properties.⁴ Special attention has been devoted to chitosan, a $\beta(1 \rightarrow 4)$ -linked copolymer of 2-amino-2-deoxy- α -glucopyranose (GlcN) and 2-acetamido-2-deoxy- α -glucopyranose (GlcNAc) obtained mainly by the deacetylation of chitin, a naturally abundant polysaccharide mainly found in the exoskeleton of crustaceans and insects.^{8,9} The appealing features of chitosan include abundant availability, biocompatibility, biodegradability, non-toxicity, low-immunogenicity, and antimicrobial activity. Additionally, chitosan exhibits mucoadhesive properties, which are beneficial to extend the retention of the drug at its application site as well as to enhance the permeation of the drug across various mucosae.^{10,11} Another important property that makes this biopolymer a potential candidate for the development of drug carrier systems is its versatility for several chemical modifications, particularly on hydroxyl ($-\text{OH}$) and amino ($-\text{NH}_2$) groups, to introduce new functionality, and to improve and/or develop a given property of chitosan.^{12,13}

Numerous chitosan-based nanodrug delivery systems including nanospheres,¹⁴ nanocapsules,¹⁵ nanomicelles,¹⁶ nanogels,¹⁷ and nanofibers⁸ have been investigated as functional drug carriers potentially useful in the treatment of diseases including cancers,¹⁴ glaucoma,¹⁸ diabetes,¹⁹ and osteoarthritis.²⁰ In this chapter, we provide an insight into the recent advances in the preparation and physicochemical aspects of chitosan-based drug nanocarriers and discuss their applications for the delivery of different therapeutic agents. We also discuss the current challenges and future perspectives on the translation of these nanomaterials to clinical applications.

4.2 Chitosan-based Drug Delivery Nanocarriers

Nanoparticles, nanomicelles, and nanogels are characterized by nanoscale dimensions and represent the most studied chitosan-based nanocarriers. Briefly, chitosan nanoparticles consist of solid systems and can be prepared in the form of nanospheres (Figure 4.1a), which correspond to a matrix-like carrier, or nanocapsules (Figure 4.1b), which correspond to a liquid core surrounded by a polymeric coating layer.²¹ Nanogels (Figure 4.1c) consist of physically or chemically cross-linked chitosan networks entrapping a large amount of water in the intermolecular space.²² The structure of nanomicelles (Figure 4.1d) is formed by a hydrophobic core and a hydrophilic shell layer that are well-suited for the incorporation of hydrophobic bioactive compounds.²³ Chitosan-based nanofibers (Figure 4.1e) correspond to structures exhibiting nanometer-sized diameters and lengths varying from micrometers to meters representing a versatile and remarkable class of 1D nanocarriers for drug delivery applications.^{8,24} In this regard, the following subsections will provide a discussion of the abovementioned chitosan-based nanocarriers highlighting their advantages and disadvantages for drug delivery applications.

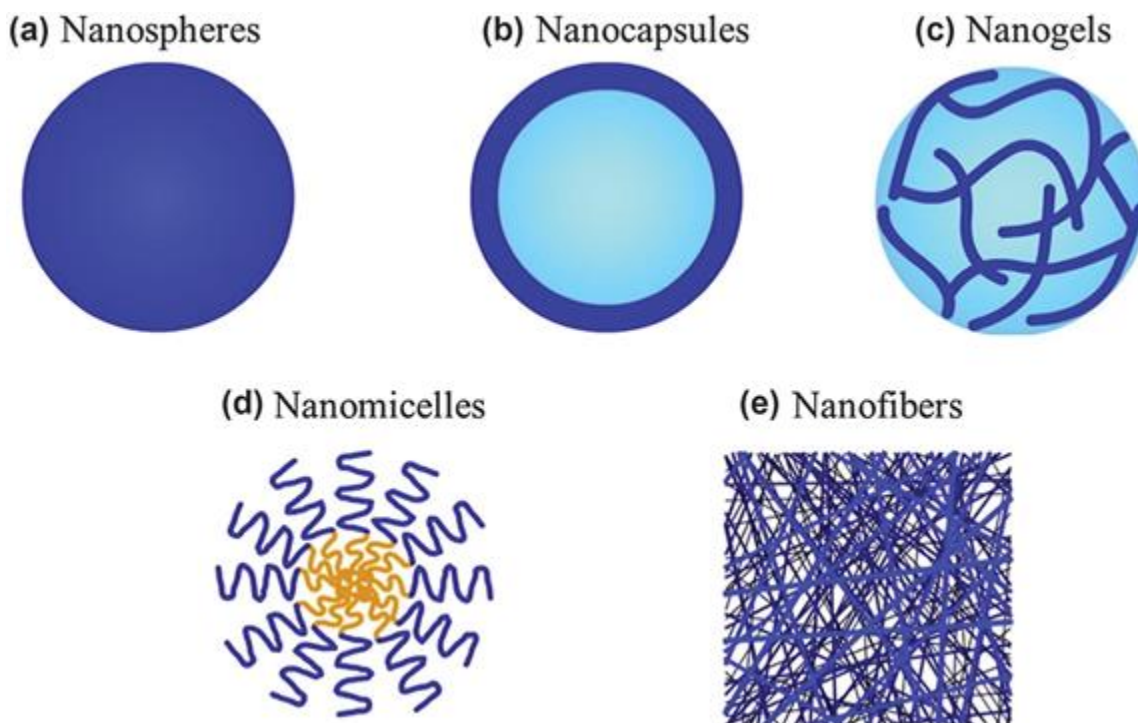


Figure 4.1 Schematic representation of chitosan-based nanocarriers: (a) nanospheres, (b) nanocapsules, (c) nanogels, (d), nanomicelles, and (e) nanofibers. The polymeric fraction is represented in dark blue. For nanocapsules and nanogels, the liquid fraction is represented in light blue. In the case of nanomicelles, the hydrophilic fraction is represented in dark blue, while the hydrophobic fraction is represented in dark blue. Adapted from ref. 21 with permission from Elsevier, Copyright 2015.

4.2.1 Nanoparticles

4.2.1.1 Nanospheres

Chitosan (CS) nanospheres are potential nanosystems for application in biomedicine in the controlled release of drugs,^{25,26} in drug signaling,²⁷ theragnostics,²⁸ treatment of wounds,²⁹ vaccine delivery,³⁰ antibacterial systems,^{31,32} among others. The synthesis of CS nanospheres can be performed by ionotropic gelation,^{33,34} polyelectrolyte complex self-assessment, reverse micellar (RMs),³⁵ and nano spray dryer,^{36,37} as illustrated in Figure 4.2. Of these, ionotropic gelation (IG) is the most used technique due to its simplicity, use of aqueous solvents, low cost, and efficiency encapsulation in terms of bioavailability.³⁸ Ionotropic gelation is

the ability of polyelectrolytes to interlock in the presence of a counterion. Tripolyphosphate (TPP) has been the most used molecule in the CS gelation process.³⁴ Parameters that must be controlled in the nanospheres preparation are the average degree of acetylation and molecular weight of the chitosan, as well as the viscosity, polymer concentration, and the cation/anion molar ratio.³⁹ Self-assessment is the aggregation of molecules with complementary charge, allowing the balance of their properties.^{40–42} The combination of CS (polycation) with other polyelectrolytes (polyanions) generates at least partial neutralization and may have regions with hydrophilic and hydrophobic characteristics that are sensitive to pH, ionic strength, and temperature.⁴⁰ Thus, CS is commonly associated with negatively-charged polyelectrolytes such as cellulose, alginate, poly(acrylic acid), pectin, carrageenan's, heparin, and ethylenediaminetetraacetic acid disodium salt (EDTA)⁴² to form complex polyelectrolytes.⁴⁰

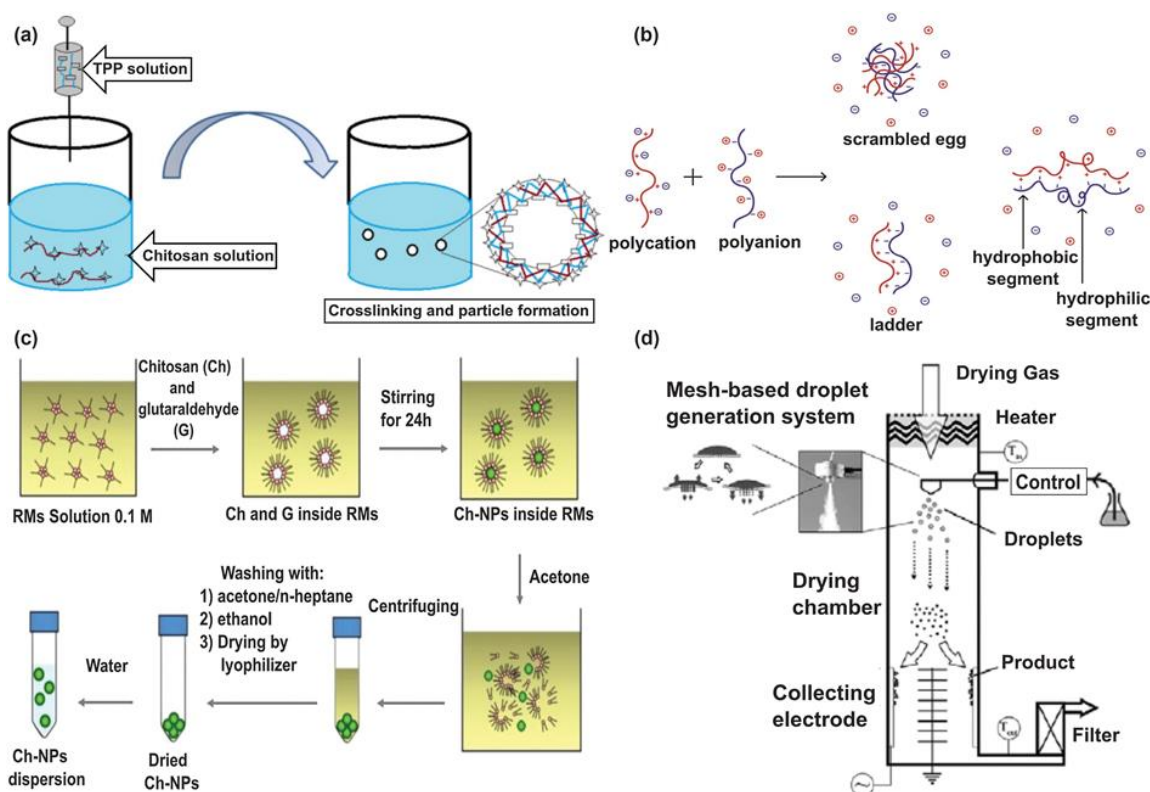


Figure 4.2 Scheme of the synthesis of CS-NEs by (a) ionic gelation by dripping TPP solution; (b) by self-assessment showing the formation of different structures with different

properties; (c) by a reverse micellar method to produce nanospheres; (d) by nano spray drying to produce nanospheres on an industrial scale. Panel (a) reproduced from ref. ⁴⁵ with permission from Elsevier, Copyright 2015. Panel (b) reproduced from ref. ⁴⁰, [10.3390/polym10030235](https://creativecommons.org/licenses/by/4.0/), under the terms of the CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>. Panel (c) reproduced from ref. ³⁵ with permission from Elsevier, Copyright 2020. Panel (d) reproduced from ref. ³⁷ with permission from Elsevier, Copyright 2010.

To produce CS nanospheres by a reverse micellar method (RM), it is necessary to use surfactant molecular aggregates to form the micelles and trap the CS inside together with a crosslinking agent, giving rise to the nanospheres. After this process, the destruction of the micelle occurs to form the nanoparticle. The advantage of this strategy is the control of the NE size from changes in the concentrations of the polymer (CS), the crosslinking agent, or the water or solvent content used.³⁵

The nano spray dryer (NSD) is a technique employed to produce nanoparticles at a high output rate, which is suitable for industrial processes. It is based on a conventional spray dryer setup, but it uses a stainless-steel membrane with holes at the microscale that vibrate to release droplets in a narrow size distribution range. This technique has the disadvantage of working at elevated temperatures, which limits the use of thermosensitive molecules.³⁷ Other synthesis routes of CS nanospheres (NE) include chemical precipitation⁴³ and solvent evaporation,⁴⁴ but these are less studied for CS-NEs.

The presence of free OH and NH₂ in CS allows chemical modifications for modulating their physical–chemical properties through the binding (grafting) of specific molecules that can play the role of signaling,²⁷ target,⁴⁶ and coating molecules,^{47,48} changing properties such as hydrophobicity⁴⁰ and mucoadhesiveness,⁴⁹ among others. This versatility allows the CS properties to be modulated to solve problems that require different properties (Table 4.1). For example, there is a difficulty in solubilizing some drugs, which leads to high dosages with low bioavailability, leading to cytotoxicity, side effects, and more invasive administrations to the body.^{45,50,51} Another problem is the half-life variations of medications due to the stimulation of enzymes released by the liver, leading to dosing errors and marked side effects. The various

physiological defense and protection mechanisms are other barriers that drugs face to reach the tissue in need of therapy.^{52,53}

Table 4.1 Characteristics and applications of CS-NEs.^a

Composition	Synthesis	Size (nm)/zeta (mV)	Drug	% EL	% EE	Findings	Ref.
NEs + glutaraldehyde	RM	134 to 327 + 40 to +60	Curcumin	2	99	– Size control	35
Cs + PEG + target (GA)	Ionic crosslink	185.3 + 15	BRUCINE	6.01	68.53	– Entrap hydrophobic molecules – Target mitochondrial – <i>In vitro</i> and <i>in vivo</i> – 4 overcoming physiological barriers	58
CS	IG	150 to 200 + 32.5	+ Bay41-4109	17.3	90.04	– Greater solubilization – Less touchiness – Oral administration – Liver cancer – 60% release in 30 hours	45
Ca-P/carboxymethyl CS + Kela (peptide)	SA	208 to 278 – 16.9 to – 26.1	DOX	3.3	90	– NEs peptide decoration – Cell HeLa – Target – Non cytotoxicity – 50% release in 25 hours	33
Oligosaccharide-arachidic	SA	130 + 13	DOX	—	53	– pH responsive – Cell FadU – 80% release in 50 hours	60
Genipin + CS-heparin	IG	250 + 32	Ciprofloxacin	45	—	– Antibacterial activity – 100% release in 9 hours	61
Carboxymethyl CS	IG	218 – 33	Carbamazepine	35	80	– Target brain – Delivery for mucoadhesion – 80% release in 10 hours	52
Galactosylated CS + oxide graphene	SA	297 – 30	DOX	~90	—	– pH responsive – 17 to 33% release in 48 hours – Hepatocarcinoma cells	62
(Di and Tri) acetate CS	Double emulsion and evaporation of solvent	< 100 – 25	DOX among others	Until 37	Until 80	– Solubility of various cancer drugs – 80% release DOX in ~3 hours	44
Glycol-CS-estrone	SA	221 to 263 + 16 to +26	Paclitaxel (PTX)	9.4	92.4	– Endocytose – Intracellular pH response – 60 to 40% release in 12 hours – Non cytotoxicity	63
CS-LDL	IG	170 + 52.8	DOX	~45	51.7	– Low cytotoxicity – Preferential uptake <i>via</i> endocytosis	50
βCD N-maleoyl-CS	IG	179 to 274 + 36 to +42	Ketoprofen (KTP)	14.8	62.6	– Solubility of poorly soluble drugs – Alta bioavailability – 30 to 60% in 24 hours	51
CS	IG	44 + 3.23	Perindopril erbumine	30.5	94.1	– Hypertension – 90% in 70 hours – Low cytotoxicity	64
CS	IG	100 + 53.8	Cytarabine	34.8	64.8	– Enhanced cytotoxicity toward breast cancer cell lines – 50% release in 24 hours	65
Dexamethasone-glycol chitosan	SA	277–289 + 15	Dexamethasone sodium phosphate	—	—	– Ocular treatment – Adhesion ocular – Safe – Anti-inflammatory efficacy – Non toxicities – <i>In vitro</i> and <i>in vivo</i> test – Non irritable – 80% release in 4 hours	41
CS–poly(<i>N</i> -isopropylacrylamide) + K237 peptide	Precipitation	125 + 19.1	Paditaxel (PTX)	11.8	—	– 60 to 70% release in 25 hours – Target	66

CS + alginate	IG	119 – 36 and +32	Amygdalin (vitamin B17)	—	90.3	– Cancer cells – Mucoadhesion – Transmigration – Anionic and cationic surface amygdalin-loaded pH responsive	67
CS	IG	223 to 507 + 27.4 to +42.7	Clindamycin	8.8	42	– Topic treatment – Target to pilosebaceous – Treatment of acnes vulgaris – About 50% release in 5 hours	68
CS	IG	~300 + 25.8	Vitamin K	3.36	78.17	– Prolong circulation	69
Phenylboronic acid + CS	SA	200–230 + 7 to +20	Curcumin	8 to 18	67 to 91	– Vt cancer – 80 to 20% release – pH responsive	42
CS + target	IG	290 + 22	Quercetin	13.01	92.04	– Paditaxel resistant lung cancer – 40% release in 30 minutes	46
CS + amide pectin	IG	130.7 + 34.6	5-Fluorouracil	—	21.4	– Greater gastrointestinal absorption – pH responsive	70
CS + hydrogel	IG	172 + 27.8	Cefepime	—	—	– Accelerates healing – 80% release in 20 hours	29
CS-BSA-Eudragit®L100	IG SA	558.2 + 12.3	Bovine serum albumin	—	90.38	– Vaccine delivery – Oral administration	30
CS	IG + NSD	160 to 1230 + 25 to +59	Amoxicillin	90	—	– Size and zeta potential controller – Antibacterial activity	71

^aEL = loading efficiency; EE = encapsulation efficiency; RM = reverse micellar; IG = ionotropic gelation; SA = self-assemble.

An efficient NE is required to present a suitable loading capacity and efficient drug encapsulation to avoid degradation during the path (before reaching the target), overcome the physiological barriers to reach the target tissue, overcome cellular barriers (if applicable), and release the drug to the therapeutic target.^{53,54} It was observed that the degree of acetylation of chitosan affects the release of bioactive molecules and may also improve the encapsulation efficiency and loading capacity of nanocarriers of water-soluble cationic drugs. This occurs due to the cross-linking between the acetate groups and divalent cations.⁴⁴

M. Xue, *et al.* investigated the effect of encapsulation of the practically insoluble hydrophobic drug BAY41-4109 ($38 \mu\text{g mL}^{-1}$ – “solubility” the Pharmacopoeia) in CS nanoparticles produced by IG with TPP as a crosslinking agent. They observed that nanoparticles of 168 ± 64 nm in size and surface charge of $+32.5 \pm 1.15$ mV had an encapsulation efficiency of 73.5% and showed a higher dissolution rate and less cytotoxicity in *in vitro* liver cells than the suspension of the drug. The release kinetics could be separated into three phases. First, a quick release of about 15% in the first moments followed by a controlled release of 40% in about 24 hours followed by a decrease in the release rate, reaching 60% of the cumulative release in 70 hours. In addition, they observed that the concentration of the drug in the plasma administered orally of NE-CS-Bay41-4109 was 5 times

greater than that of Bay41-4109 and the bioavailability was 70.28%, about 4 times greater than the suspension of the drug.⁴⁵

X. Hou, *et al.* also made efforts to tackle the problem of poorly soluble drugs, where they encapsulated Ketoprofen (KTP) in *N*-maleoly CS NEs grafted in beta-cyclodextrin (CD). NEs presented a size range of 179–274 nm and zeta potential of 36.2–42.4 mV with 14.8% loading efficiency. The rate of KTP release was rapid in the first 2 hours followed by a controlled release up to 24 hours. Significantly greater bioavailability of NEs CD-*g*-CS was observed in relation to NEs of CS.⁵¹ J. Tian, *et al.* developed NEs of CS and LDL extracted from rats displaying a size of 179 nm and zeta potential of +48.3 mV by self-assembly in acidic solution (pI 6.5) for controlled release of doxorubicin hydrochloride (DOX). DOX encapsulation in CS-LDL NEs caused less toxicity due to preferential endocytosis of gastrointestinal cancer cells. This occurs owing to its negative surface charge causing autophagy in up to 24 h, which is significantly higher than suspended DOX.⁵⁰ It is worth highlighting the importance of modulating the surface load of CS-NEs for reaching the therapeutic target. These results show that the loading of drugs in CS-NEs can decrease the dose administered in treatments and make administration *via* less invasive routes feasible due to the bioadhesion properties of CS-MPs.

The bioadhesion of CS NEs is consolidated in the literature.⁴⁹ Yu *et al.* used this property to evaluate the safety of the administration of glycol-CS-NEs produced by self-assessment in the delivery of dexamethasone for ophthalmological applications. In *in vitro* tests the authors observed low cytotoxicity and the capacity of cell adhesion. In addition, they did not observe eye irritation or significant changes in eye pressure and proved the permanence of NEs *in vivo* tests in the eyes of male rats after 2 hours of administration.⁴¹ Liu *et al.* showed the role of adhesion of carboxymethyl-CS-NEs (size = 218 nm and zeta potential = −33 mV) produced by the solvent evaporation method loaded with carbamazepine (CBZ) to reach the brain *via* the nasal route,⁵² which is a path already explored in the literature.⁵⁵ The CBZ release rate was well sustained, releasing 20% in about 1 hours, 40% in 3 hours, 70% in 7 hours, and 87% in 24 hours. They observed that the plasma and cerebral CBZ bioavailability increased 2.6 and

8.1 times that of CBZ-NEs in relation to CBZ-Solution, respectively. In addition, the concentration of CBZ in the brain remained significantly higher for 240 min. These results showed the bioadhesion of NEs from carboxymethyl-CS to the mucosa with a significant effect on the uptake of the drug by the mucosa.⁵²

The administration of vaccines through routes less invasive than intraparental ones is desirable due to the ease of application, lower costs, and reducing rejection in the population.^{56,57} B. Xu, *et al.* developed a Manoses-CS-BSA-Nes (size = 558 nm and zeta potential = 12.3 mV) coated with Eudratgit[®] L100 (Eud) to assess the immune response of the orally administered vaccine. Eudragit has the function of protecting Nes in the gastrointestinal system. CS had an important role in mucosal adhesion and migration, followed by the binding of mannose to dendritic cells and macrophages and generation of the immune response.³⁰

The majority of drugs have their target locations at the cytosol or in organelles, and their efficient delivery to these locations is the key to achieving a maximum effect with minimum side effects.⁵⁴ Z. Chen, *et al.* produced multifunctional NEs (MNEs) associating *N*-quaternary ammonium-CS (NQC) and *N*-glycyrrhetic acid–polyethylene glycol (PEG)–CS (NGPC) loaded with brucine (anti-cancer drug), which was surface modified with glycyrrhetic acid (GA) (target). These MNEs (size = 183 nm) were able to efficiently encapsulate the drug brucine (efficiency encapsulation (EE) = 68.5%). In addition, the PEG and GA that were present on the surface of the MNEs performed the coating/protection functions of the MNEs until reaching the acidic tumor environment causing endocytosis, respectively. After endocytosis, MNEs could absorb protons (proton sponge effect) until the endosome ruptures, causing an increase in the surface charge of MNEs that contributes to targeting negatively charged mitochondria to the action of brucine.⁵⁸

CS-NEs contribute to the bioavailability and efficient delivery of drugs safely. Despite this, investigations focusing on the drug delivery at specific targets are in their infant stages and have a long way until real applications can be reached. Thus, it is necessary to further explore NEs loaded with target molecules, in addition to the drug, which can be administered by non-invasive routes. Such systems are friendly to the physiological system to

achieve the therapeutic function with the right dosage at the right location. In addition, large-scale production of such systems has yet to be explored. For instance, the production of CS by Nano Spray dry, which is an established industrial technique, produces particles in the range of 1 to 10 μm .^{37,59}

4.2.1.2 *Nanocapsules*

Nanocapsules (NCs) are spherical particles exhibiting a core-shell morphological structure with a liquid/solid core that is coated with a polymeric shell, which is absent in nanospheres.⁷²⁻⁷⁴ For drug delivery, they must have a high capacity to load active compounds, combined with biodegradability\biocompatibility.⁴ In general, the release can occur by diffusion of the drug through the matrix, diffusion of the drug from the shell, breakdown-erosion of NCs, and a combination of erosion-diffusion processes.⁷⁵ NCs for drug delivery must be soluble in physiological pH, protect the drug from degradation as well as afford optimal release kinetics, and exhibit adequate size for the application and loading capacity.⁷⁶

There are two main techniques for the preparation of NCs: solvent evaporation and emulsion polymerization. In the solvent evaporation method, a polymeric emulsion is prepared in a volatile organic solvent (chloroform, dichloromethane, ethyl acetate, *etc.*) by high-speed homogenization for mixing the substances to produce oil-in-water-in-oil interfaces.⁷⁷ The emulsion obtained is evaporated under reduced pressure and with continuous stirring, then the NCs are purified by repeated centrifugation and washing with deionized water.^{4,75}

In the emulsion polymerization method, the main components for polymerization in the presence of surfactants are water, surfactants, and water-soluble initiators for polymerization.^{78,79} The initiation of polymerization is triggered by free radicals,⁸⁰ which are usually generated by initiator molecules or by irradiation with UV light. Typically, these NCs have a narrow size distribution.⁷⁵ In the absence of surfactants, emulsifier-free⁸¹ approaches can be employed using initiator and water-soluble monomers,^{82,83} in which the ionization of the initiator triggers the polymerization forming NCs,⁸⁴⁻⁸⁷ as illustrated in [Figure 4.3](#)

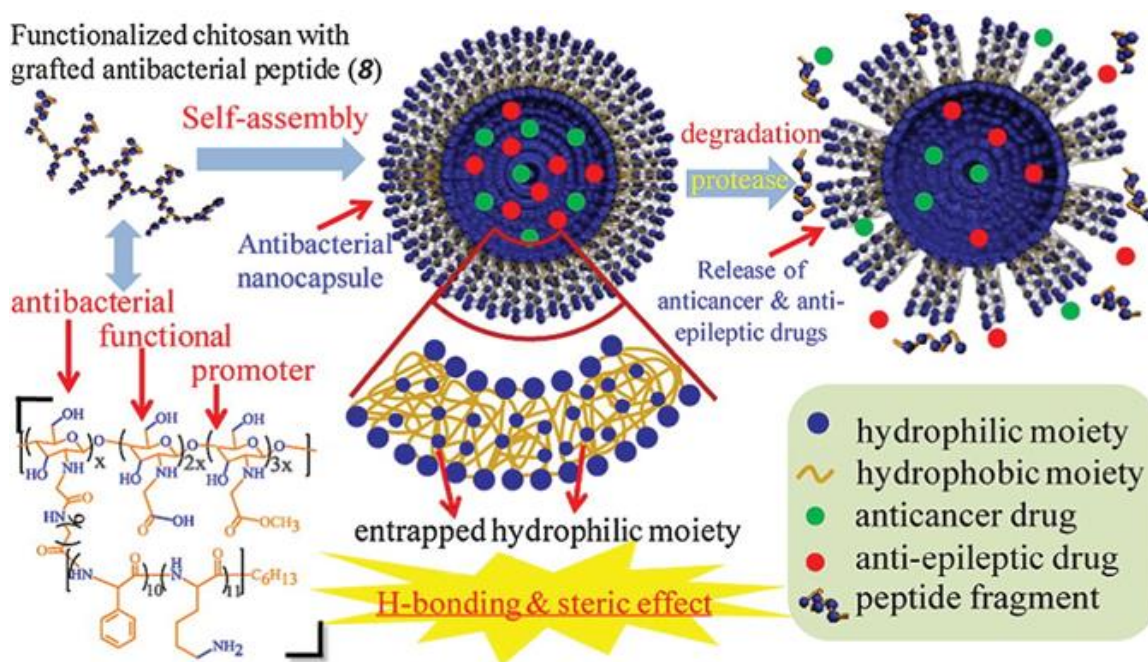


Figure 4.3 A polypeptide-grafted chitosan-based nanocapsule provides excellent antibacterial efficacy and can deliver anticancer and antiepileptic drugs simultaneously. The membrane of the nanocapsule is fabricated of hydrophobic and entrapped hydrophilic moieties. Drugs were released faster in the presence of protease by biodegradation of the polypeptide. Reproduced from ref. 86 with permission from American Chemical Society, Copyright 2013.

Nanotechnology has been continuously improving the design of NCs that reduce the dose of administered drug, avoiding contamination of healthy tissue and allowing its use by high-risk patients. The design studies of chitosan nanocapsules (CS-NCs) reduce the administration time of drugs that are extremely harmful to the human body (inducing severe side effects) and are often difficult to administer. One of these molecules is Bedaquiline, which is a drug used to treat *Mycobacterium tuberculosis* (TB), with serious consequences for human health. In the work of De Matteis *et al.*,⁸⁸ the encapsulation of Bedaquiline in chitosan-based nanocapsules was produced by an emulsion polymerization method (size: 328 ± 35 nm and Z-potential: $+26 \pm 4$ mV),⁸⁸ which showed high drug loading values. Furthermore, because CS is rich in amino groups, it favored the interaction of the NCs with the mucosa of the respiratory tract, reducing the biodistribution, administration dose, and adverse effects on the respiratory system.^{17,25} In

vitro Bedaquiline release profiles showed 40% release after 3 days of incubation in TB cultures. No cytotoxic effects were found in A549, HepG2, and THP-1 cell lines at the concentration necessary to kill bacteria. The authors concluded that these nanocarriers are excellent candidates for safe administration *in vivo*.⁸⁸

Many molecules have poor solubility in water, which causes problems in dermatological treatments due to their deficient penetration into the skin. This problem was investigated in the production of chitosan-Pluronic F127 nanocapsules (size: 60 ± 20 nm, Z-potential: +20 mV) using a nanoprecipitation method, which did not require chemical crosslinking between these biocompatible polymers.⁸⁹ CS in this system can penetrate the dermis and epidermis due to its cationic properties,⁹⁰ while Pluronic F127 is a charger for hydrophobic molecules, facilitating the solubility of molecules in transdermal release.⁸⁹ These nanocapsules carried cyclosporin A (CsA), which is a modulating fungal metabolite in hair growth. The *in vitro* study of the penetration of the NCs into the skin showed that greater permeability depends on the molecular weight of the chitosan, where 10 kDa is the optimal value for the formation of the NCs for transdermal release of hydrophobic drugs.^{77,89} *In vivo* studies of the efficacy of the CsA-loaded nanocapsule for hair growth were studied using a mouse model, and the study showed that the system induced an anagen phase for hair growth through the release of CsA.⁸⁹

4.2.2 Nanogels

Nanogels (NGs) are hydrogel nanostructures based on physical–chemical interactions between cross-linked polymers, with structures ranging typically from 20 to 200 nm.⁹¹ Such materials present optimal biodegradability and biocompatibility properties for internalization in the human body. Besides, their large specific surface area allows functionalization and bioconjugation suitable for drug-delivery applications.^{22,92} The small size and the cross-linked structures are fundamental characteristics for drug delivery because it allows the NGs to be modulated according to the water or hydration content. Moreover, the active substances can be protected from the external environment to be released when the NGs experience appropriate physicochemical

conditions.^{93,94} The release response of these substances is fast compared to microgels and hydrogels.⁹¹

It is well-known that chitosan-based nanogels exhibit an outstanding hydration capacity due to the presence of functional groups such as amine ($-\text{NH}_2$), and hydroxyl ($-\text{OH}$)^{22,95} typical of chitosan.⁹⁶ Drug delivery occurs when the nanogel is disturbed by changes in pH, temperature, irradiation, magnetic field, and ionic concentration. Chitosan nanogels (CS-NGs) exhibit mucoadhesive and bioadhesive properties by their cationic characteristic,^{91,94,97} which helps to overcome epithelial and intermural barriers, thus improving the treatment efficacy. CS-NGs can encapsulate therapeutic biomolecules such as drugs,⁹⁸ genes,⁹⁹ proteins,^{100,101} and ultrasound contrast agents.^{102–104} For example, encapsulating nucleic acids that have a negative charge is easier to do by designing suitable polyelectrolyte nanogels, a problem that is not solved with macroscopic hydrogels,^{103,105} as illustrated in [Figure 4.4](#).

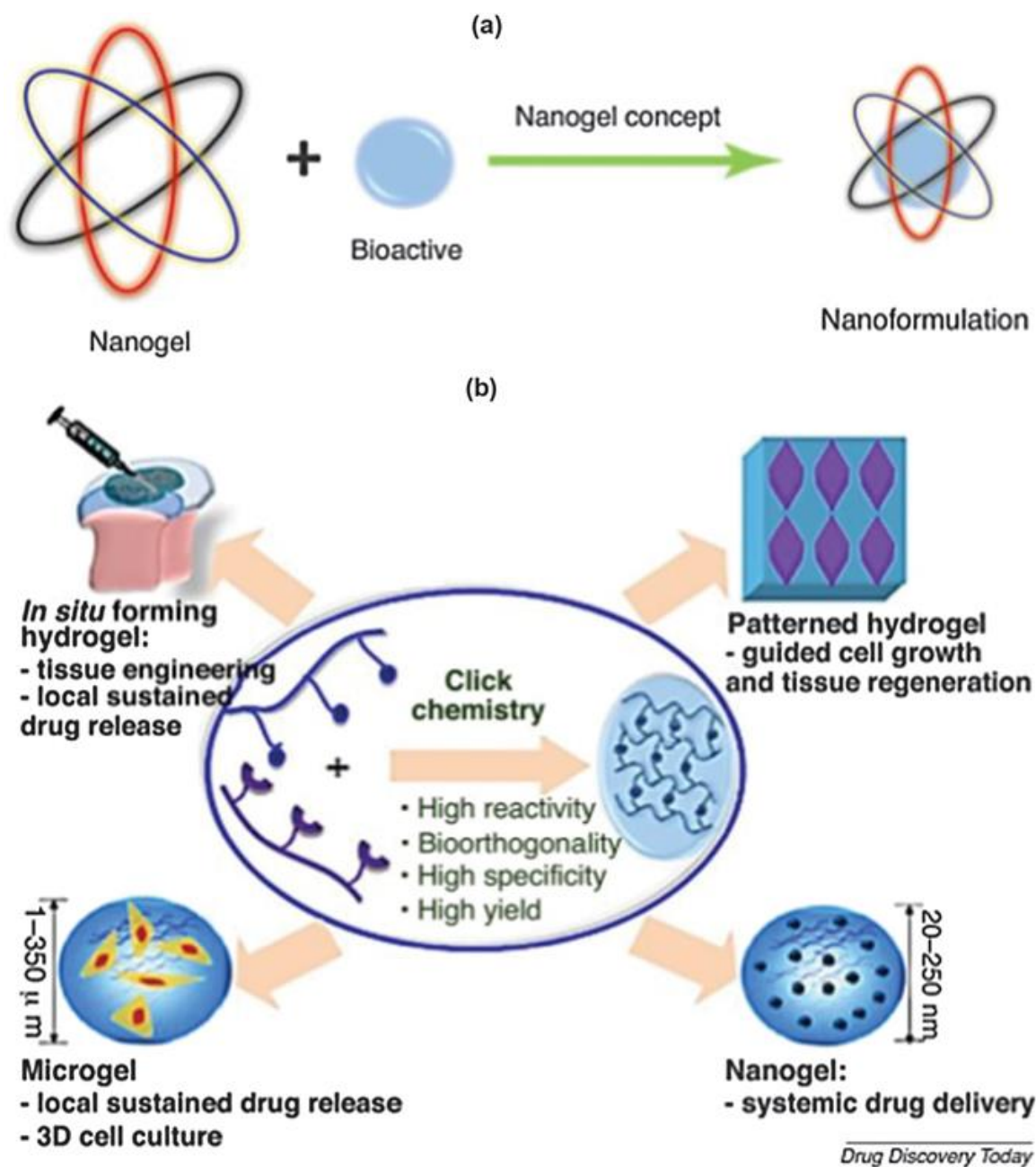


Figure 4.4 Fundamental concept. (a) NGs are cross-linked structures with biocompatible characteristics for drug delivery with outstanding modulation according to the water/hydration content and physiological environment. (b) Chemical preparation as click chemistry is a straightforward approach to form nanogels with varying dimensions with high reactivity, biorthogonality, specificity, and selectivity for drug release in a wide range of biomedical applications. Reproduced from ref. 103 with permission from Elsevier, Copyright 2018.

NGs can be activated by remote stimuli or in specific conditions upon interaction with irradiation,¹⁰⁶ metallic and biomolecules materials,^{107,108} thermo-responsivity,¹⁰⁹ as well as under pH changes.¹¹⁰ The result is the activation of its properties by changes that occur in the polymeric crosslinks that forms the nanogel,²² which triggers the release of the active substance.^{111,112} Other types of nanogels release the active substance at the same time as they biodegrade in the physiological environment.^{110,113} NGs can form by crosslinking between polymers using different physicochemical methods: The self-assembly method is a physical process for the nanogel formation, in which mixed polymers are combined by non-covalent interactions such as hydrophobic–hydrophobic, hydrophilic–hydrophilic, ionic interactions, van der Waals, and hydrogen bonds.^{22,95} Chemical cross-linking methods for nanogel formation occur by the association of polymeric chains by covalent bonds of the polymers' functional groups, generating stable systems.^{95,114} This process can keep drugs stable inside the cross-linked system and release them at physiological pH. However, for biomolecules such as proteins and genes, they can generate denaturation by the synthesis process. The synthesis of NGs by heterogeneous polymerization of monomers in an aqueous phase generates colloidal systems but is not compatible with biomolecules.^{115,116} Another nanogel fabrication technique uses an *in situ* process where biocompatible water-miscible solvents are mixed with polymers that dissolve in this emulsion. During this process, the active substance (proteins, genes, nucleic acids, and drugs) is added to the compound, yielding the final nanogel.^{22,117} These nanosystems have gained importance for drug injectable administration due to the capacity of controlled deposition of the active substance at specific sites. Nanofabrication is another interesting method for manufacturing NGs due to the ability to control their formation with optimal properties for drug delivery.^{118,119} Examples include the growth of NGs generated by photolithography,^{120,121} microfluidic devices,¹²⁰ and 3D printing.¹²² Photolithography is a method using light to transfer patterns from a photomask to a light-sensitive chemical (photoresist) on a substrate and selectively remove parts out of the substrate, and this process needs a strictly environmentally controlled space.¹²¹ As mentioned by Kaur *et al.*,¹²³ at first it was necessary to release

the cross-linkable polymer on a pre-baked photo resistant layer, then the polymer was molded on a silicon wafer by using appropriate patterns and then kept in contact on UV light and all residues were eliminated. Subsequently, oxidation was carried out using oxygenated plasma and the micro–nano gels were collected by dissolution for biomedical applications.¹²⁴ Microfluidics for NGs synthesis are usually built on the rapid mixing of a solution containing polymers and biomolecules with a miscible non-solvent of the polymers,¹²⁵ being a straightforward procedure and reproducible.¹²⁶ 3D printing can revolutionize the traditional fabrication of NGs by improving their biomedical properties.¹²⁵ Basically, this technique can print drug/photoinitiator-loaded substances in hydrogel matrices.¹²²

CS-NGs in drug delivery applications provide a cross-linked system to load substances, avoiding unwanted release, in addition to offering protection of the substance against degradation at physiological pH.^{22,95} Furthermore, the nanogels exhibit a high hydration capacity, which allows their transit through the blood circulation without affecting the capillaries, since the NGs can modify their own morphology by expanding or shrinking to lodge in affected tissue.^{92,127}

As mentioned before, NGs can be designed to be activated by changes in pH produced by the pathological tissue. For example, one of these nanogels combines *N*-lysinal-*N'*-succinyl chitosan (NLSC), poly(*N*-isopropylacrylamide) (pNIPAA), and bovine serum albumin (BSA).⁹⁶ BSA is a biocompatible macromolecule with negative charges that interacts with NLSC, increasing the stability of the nanogel during the circulation and swelling in the acidic environment of the pathology, releasing the drug.¹²⁸ In intravenous administration, these NGs accumulate at the tumor tissue site by the enhanced permeability and retention (EPR) effect, then internalize into endosomes and lysosomes (endo-lysosomes). At the endo-lysosomes the pH (4–6) is lower than the pI of NGs, where the amino groups protonate producing electrostatic repulsion between polyelectrolytes and triggering the swelling of the nanogel. This rapid release of the encapsulated drug kills the cancer cells.¹²⁸

In photothermal therapy, NGs have been employed for destroying human macrophages that generate inflammation, a consequence of bacterial

contamination.¹¹² These NGs can be designed from the interaction of hyaluronate–chitosan to obtain negative charges on their surface, improving biocompatibility. These systems can encapsulate therapeutic agents with anionic photosensitizers (PS) (tetra-phenyl-porphyrin-tetra-sulfonate (TPPS4)), where the PS interacts with cellular oxygen to form reactive oxygen species (ROS) that oxidize amino acids and other biomolecules, inducing cell death.¹²⁹ These NGs have been used in *in vivo* studies for photodynamic therapy of inflammatory diseases of articular joints using the murine animal model of antigen-induced arthritis (AIA). In the work of Schmitt *et al.*,¹³⁰ the NGs were irradiated at 652 nm at 25 J cm⁻² to break the electrostatic interaction of the polyelectrolytes in the nanogel by activating the photosensitizer. In this process, hyaluronate and PS molecules were absorbed by macrophages, cellular organelles, but not by the nucleus, which reduced joint inflammation in the murine model of rheumatoid arthritis.¹³⁰ To verify the efficiency of this nanosystem in photodynamic therapy protocols, macrophages were exposed for 24 h to NGs and irradiation at 652 nm at a dose of 0.5–15 J cm⁻² showed cell survival, which is suitable for this type of treatment.¹³⁰

For the treatment of cutaneous wounds, Ashoori *et al.*¹³¹ reported the fabrication of CS-NGs *via* ionic gelation of CS employing a polyanion, sodium triphosphate (TPP), which were loaded with probiotic agents *Lactobacillus reuteri*, *Lactobacillus fermentum*, and *Bacillus subtilis* sp. *Natto*. Such probiotics have beneficial properties for human health, since they modulate the immune system by inhibiting the proliferation of pathogens, reducing inflammation, and inducing wound healing.^{131,132} These nanogels were used for the treatment of wounds (pH: 7.9–8.9) generated in an animal model, where the pH of the wound caused the deprotonation of the amino groups of CS, allowing the release of probiotics to influence wound healing. This behavior can be attributed to the synergistic effects of polysaccharides and probiotics, which induce the deposition of proteoglycans and angiogenesis, rapidly improving healing by reducing the inflammation. The wound healing studies showed excellent results in two weeks of treatment.¹³¹

4.2.3 Nanomicelles

Micelles are self-assembled structures that exhibit a core-shell configuration in an aqueous environment. In general, the internal core is composed by hydrophobic segments surrounded by hydrophilic segments.^{133,134} These structures are obtained from chain-like amphiphilic molecules, which have both hydrophilic (head) and hydrophobic (tail) functional groups.¹³⁵ In order to obtain micelles, amphiphilic molecules are added in the water until it reaches the critical micelle concentration (CMC), when the limit of their solubility is achieved. Above this concentration, the molecules added tend to organize themselves into micellar aggregates.^{135,136} It is also possible to obtain polymeric micelles (PM), as displayed in [Figure 4.5a](#), which are composed of a different amphiphilic graft or block copolymers.^{137,138} As an example of polymeric micelles, a TEM micrograph of PTX-loaded *N*-octyl-*N*-(2-carboxyl-cyclohexamethenyl) chitosan micelle¹³⁷ can be seen in [Figure 4.5b](#).

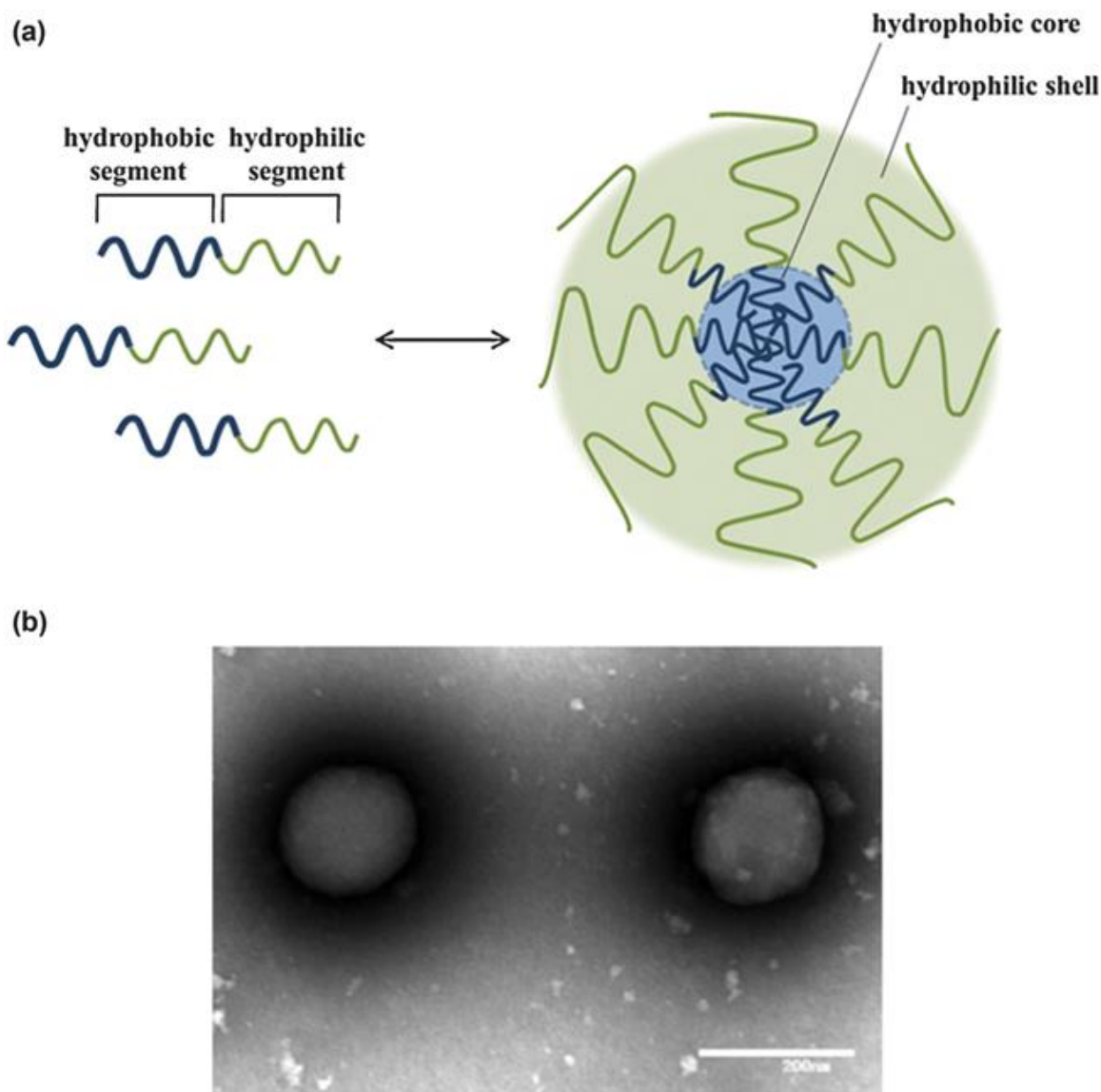


Figure 4.5 (a) Scheme illustrating the self-assembling structure polymeric micelle, composed by a hydrophobic core and a hydrophilic shell. (b) TEM of PTX-loaded *N*-octyl-*N*-(2-carboxyl-cyclohexamethenyl) chitosan micelles. Panel (a) adapted from ref. [139](#) with permission from Elsevier, Copyright 2012. Panel (b) reproduced from ref. [137](#) with permission from Elsevier, Copyrights 2011.

PMs are one of the most promising structures for drug delivery systems due to their capability to physically incorporate hydrophobic drugs into the hydrophobic core, thus enhancing the solubility of these compounds in aqueous medium.^{[140–142](#)} Compared with the amphiphilic low-molecular-

weight molecules, the polymers exhibit slower dissociation and a lower critical micelle concentration (CMC), which assists the drug stability.^{133,143}

The process of self-assembling polymeric units in order to form micelles is thermodynamically favorable, in which the hydrophobic and hydrophilic blocks are arranged toward minimizing the free energy of the system.¹⁴⁴ Above the CMC concentration, the hydrophobic blocks are attracted, avoiding direct contact with water, thus resulting in the formation of the core of the micelle.¹⁴⁵ The hydrophilic chains remain interacting with the water, forming a shell around the hydrophobic core, which helps to stabilize the core-shell structure.^{145,146} As mentioned before, this structure enables the encapsulation of hydrophobic drugs into the hydrophobic core.¹⁴⁷ There are many methods that can be used to prepare drug-loaded polymeric micelles, such as direct dissolution, dialysis, oil-in-water emulsification, solvent evaporation, and freeze-drying.^{148,149}

Direct dissolution is the simplest method for polymeric micelles preparation.¹⁴⁵ In this method, the polymer at a concentration higher than the CMC as well as the drug are dissolved in an aqueous solvent, where hydrophobic drugs are preferentially loaded into the hydrophobic core of the resulting micelles.¹⁵⁰ Despite being a simple and widely used method, it may present low drug loading, which can be improved by heating the solution.¹⁴⁶ Varshosaz *et al.* synthesized biotin-chitosan-PMVEMA (poly(methyl vinyl ether-*alt*-maleic acid) micelles for delivery of doxorubicin (DOX) using the direct dissolution method. For this purpose, DOX and biotin-chitosan-PMVEMA were dissolved in deionized water and then the system was stirred at 50 °C.¹⁵¹ In general, changes in temperature of the system can affect the efficiency of encapsulation of the drug due to micellar growth.¹⁵² Besides, the temperature and concentration of the polymer impact the free energy of solubilization (ΔG°); thus, the increase in the concentration/temperature leads to more negative values of ΔG° , which indicates that the solubilization of the drug occurs spontaneously.¹⁵²

One of the most used methods found in the literature to produce chitosan micelles relies on the dialysis process.^{143,153–155} In this method, the polymer and drug are dissolved in an organic common solvent (dimethylsulfoxide, *N,N*-dimethylformamide, acetonitrile, tetrahydrofuran,

acetone, or dimethylacetamide),¹⁵⁶ and then the system is dialyzed against water, in which the organic phase is slowly removed, triggering the micellization.¹³⁹ Among the chitosan derivative micelles produced by this technique, one can cite the work of Almeida *et al*, who prepared camptothecin-loaded mPEG–chitosan–oleic acid polymeric micelles. First, they dissolved mPEG–chitosan–oleic acid polymeric (mPEG–CS–OA) in DMSO : HCl 0.1 M and kept it under stirring. Then, the camptothecin previously dissolved in DMSO was added to the mPEG–CS–OA. The resultant mixture was dialyzed against water to remove the DMSO and free drug.¹⁵⁷

In oil-in-water emulsification, the hydrophobic drug and the polymeric micelles are dissolved in a water-immiscible organic solvent (*e.g.*, chloroform or methylene chloride), the oil phase. Then, this phase is added to an aqueous phase under vigorous stirring, forming the O/W emulsion. After solvent evaporation, a free dispersion micelle-drug is obtained.^{146,149} Another possibility is to produce the micelles by another technique and encapsulate the drug by oil-in-water emulsification as suggested by Woraphatphadung *et al*. They developed *N*-naphthyl-*N,O*-succinyl chitosan micelles for oral meloxicam delivery; the micelles were firstly produced by dialysis, then the meloxicam was solubilized in dichloromethane and mixed with the aqueous solution containing the micelles under constant stirring.¹⁵⁸

The solvent evaporation method is based on dissolving the polymer and the drug in a volatile organic solvent, such as methanol, ethanol, and acetone, where a thin film of drug/polymer is obtained after the evaporation of the organic solvent. The reconstitution of the film in an aqueous phase under stirring leads to the production of drug-loaded micelles.^{144,149} For example, Yang *et al*. developed a delivery system based on L-carnitine (LC) conjugated chitosan (CS)–stearic acid (SA) polymeric micelles for encapsulation of paclitaxel (PTX). In this study, PTX was dissolved in methanol, while CS–SA and LC–SA were dissolved in purified water. Then, PTX solution was added dropwise into the LC–SA/CS–SA solution. After evaporation of the solvent, a film was formed and redispersed in water, resulting in a drug-loaded micelle.¹⁵⁹

In the freeze-drying technique, both drug and polymer are dissolved in a water/*tert*-butanol mixture and the solution is freeze-dried.¹⁵⁶ The resultant

solid is added into an aqueous medium and the drug-loaded polymeric micelles are formed.¹⁴⁶ Meanwhile, freeze-drying is usually combined with other techniques described above to obtain long-life injectable formulations, which commonly happens with chitosan micelles.¹⁴⁹ For example, Zhang, *et al.*¹⁵⁵ prepared paclitaxel-loaded *N*-octyl-*O*-sulfate chitosan micelles using a dialysis method, and the resultant micellar solution was freeze-dried and kept in a refrigerator at 4 °C before use. According to the authors, the lyophilized powder could be reconstituted easily in an aqueous environment even after 2 months, showing good stability.¹⁵⁵

The properties of chitosan and its derivative polymeric micelles make them suitable for a wide range of applications as drug delivery systems.^{160–164} Several studies have demonstrated the application of these systems in cancer treatments. For instance, amphiphilic benzoic-imine-containing PEGylated chitosan/4-(dodecyloxy)benzaldehyde (DBA) micelles containing indocyanine green (ICG) exhibit great potential for cancer theranostic.¹⁶⁵ Qu *et al.*, have developed anisamide-conjugated *N*-octyl-*N*,*O*-maleoyl-*O*-phosphoryl chitosan (a-OMPC) micelles for paclitaxel (PTX) delivery, and this system featured promising potential for anti-prostate cancer treatment.¹⁶⁶ Another application was reported by Kim *et al.*,¹⁶⁷ who developed a hydrophobically modified glycol chitosan (HGC) polymeric nanomicelle loaded with the immunosuppressive agent tacrolimus (TAC) to enhance its renal delivery. The results revealed that HGC-TAC nanomicelles are non-toxic. Besides, with a single intravenous administration it was possible to achieve a therapeutic efficacy comparable to that obtained from daily intraperitoneal injections of unencapsulated TAC for 14 days, without any systemic side effects.

The micelles can also be used as wound healing systems. For this purpose, Negi *et al.* prepared thymoquinone-loaded chitosan-lecithin micelles, which enhanced the delivery of the drug to the wound bed.¹⁶⁸ Finally, one can also mention the application as mucosal drug delivery, as demonstrated in the study of Le-Vinh *et al.*, who prepared phosphorylated chitosan-stearic acid micelles (CSSAP) with a zeta potential changing property. These micelles showed effective mucus permeation and cell association, as a promising platform for mucosal drug delivery.¹⁶⁹

4.2.4 Physicochemical Characterization of Nanospheres, Nanocapsules, and Nanogels

Different characterization techniques have been applied to evaluate the physicochemical properties of nanospheres, nanocapsules, and nanogels. Dynamic light scattering (DLS) is a technique used to determine the size distribution of nanostructures in solution. It can also be designed to scan the pH and characterize the response of the structural changes that occur in nanogel cross-linking.^{170,171} This measurement can be accompanied by the zeta potential, which provides information on the surface charge of the nanostructure, in addition to evaluating how the nanostructures behave at physiological pH for drug delivery applications.^{93,100}

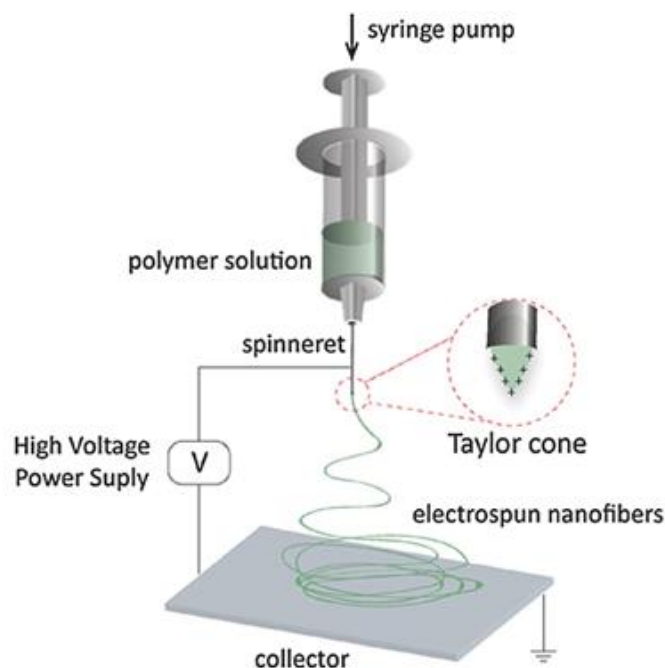
Microscopy techniques including atomic force microscopy (AFM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) provide information on the structure, size, and morphology of the nanocapsules and on the encapsulation of the active substance.^{172–175} X-ray photoelectron spectroscopy (XPS) is also used for the surface chemical characterization of the nanostructures.⁸⁵ The conformation and chemical interaction of the polymers and active substances in the nanostructures can be characterized by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance, which allows investigation of the drug delivery nanosystem.^{131,171,176} Thermal analysis such as differential scanning calorimetry (DSC) provides details on the nanostructure formation or association of the drug with the polymeric structure, relating the peaks of each compound to the change of temperature.^{91,177} Spectrophotometric techniques (*e.g.*, UV-VIS,^{88,178} fluorescence^{139,150}) and chromatographic (*e.g.*, high-resolution liquid chromatography (HPLC)¹⁶⁷) methods are commonly used to measure the concentration of the bioactive compounds incorporated into chitosan-based nanostructures and to evaluate their release profile in different medium.

4.2.5 Nanofibers

4.2.5.1 *Electrospun Nanofibers*

Electrospinning (ES) is an electrohydrodynamic process employed in the production of nano–microfibers. The basic and main components used in ES are the high-voltage power supply, a grounded conductive collector and a solution container with a spinneret, as illustrated in [Figure 4.6a](#). In this technique, an electric field is applied to the polymeric solution or melt, producing a polymeric jet with its consequent elongation. The polymeric jets produced are stretched, the solvent is evaporated, and the nanofibers are then deposited on the metallic collector.²⁴

(a) Electrospinning



(b) Solution blow spinning (SBS)

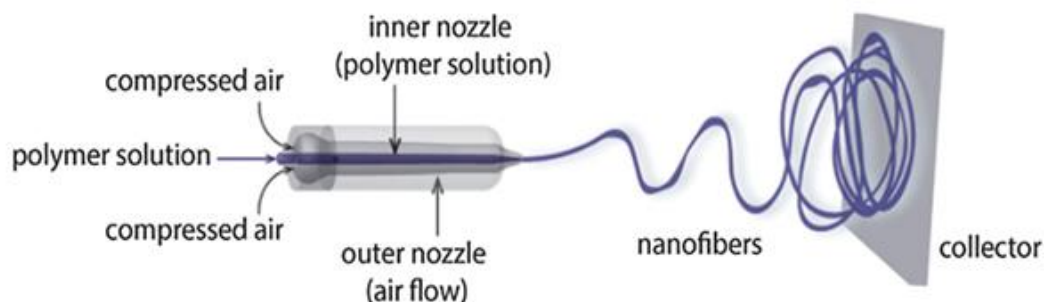


Figure 4.6 Schematic representation of electrospinning (a) and solution blow spinning (SB-spinning) apparatus (b). Panel (a) reproduced from ref. [179](#) with permission from

A range of parameters can affect the ES process, namely: (i) solution parameters such as viscosity, molecular weight, conductivity, concentration; (ii) processing parameters such as polymer solution feed rate, type of needle or absence of needle, applied voltage, distance between spinneret, and collector, and (iii) environmental parameters such as temperature and humidity. These parameters influence the morphology, orientation, and quality of nano–microfibers, contributing to several characteristics such as high surface to volume ratio, permeability, stability, porosity, flexibility in assembled structure, and versatility in the materials and drugs formulation.¹⁷⁹ Herrero-Herrero and collaborators¹⁸¹ used PLA and PCL electrospun fibers to encapsulate BSA and investigate the electrospinning parameters and their influence on fiber morphology and controlled release. The results showed that the solvents used in the production of electrospun and the diameter of the fibers produced influenced the controlled release of BSA.¹⁸¹ The combination of materials allowed the development of composite nanofibers with improved electrical, mechanical, biomedical, and optical properties to be applied in areas such as sensors,¹⁸² biosensors,¹⁸³ and controlled release.⁸ Among these materials, chitosan has been used in the production of nanofibers by ES, being used in several applications such as wound dressing,^{8,184–186} active food packaging,^{187–189} tissue engineering,^{190–192} and water purification.^{193–196}

Qin and co-workers¹⁹⁷ presented nanofiber films with rapid oral dissolution prepared with chitosan and pullulan using the ES technique. The results showed the complete dissolution of the nanofiber films in 1 min. Aspirin was used as a model drug due to its low solubility in water and showed rapid release when encapsulated into nanofiber films, confirming its potential use in mucosal-oral delivery systems.¹⁹⁷ In another work,¹⁹⁸ a bilayered electrospun membrane was produced with suitable properties for wound dressing applications. The upper layer of the membrane was composed of nanofibers of hyaluronic acid and polycaprolactone, while the bottom layer was composed by chitosan and zein nanofibers with salicylic acid. The membrane showed anti-inflammatory, anti-microbial, and appropriate mechanical properties.¹⁹⁸ Dos Santos *et al.*⁸ used coaxial

electrospinning to produce chitosan nanofibers in the shell layer and PVA and tetracycline hydrochloride (TH) as a core layer ([Figure 4.7a](#)) in which the effects of the average degree of deacetylation of chitosan and of the post-electrospinning genipin crosslinking on the physicochemical, biological, and drug release profile were evaluated. The developed system was applied as a drug delivery platform for periodontitis treatment. Cross-linked nanofibers exhibited a sustained release of TH over 14 days as well as displayed cytocompatibility toward fibroblast (HDFn) cells. Regardless of their average degree of deacetylation, only structures containing TH exhibited antibacterial activity against a wide range of periodontal pathogens ([Figure 4.7b](#)).

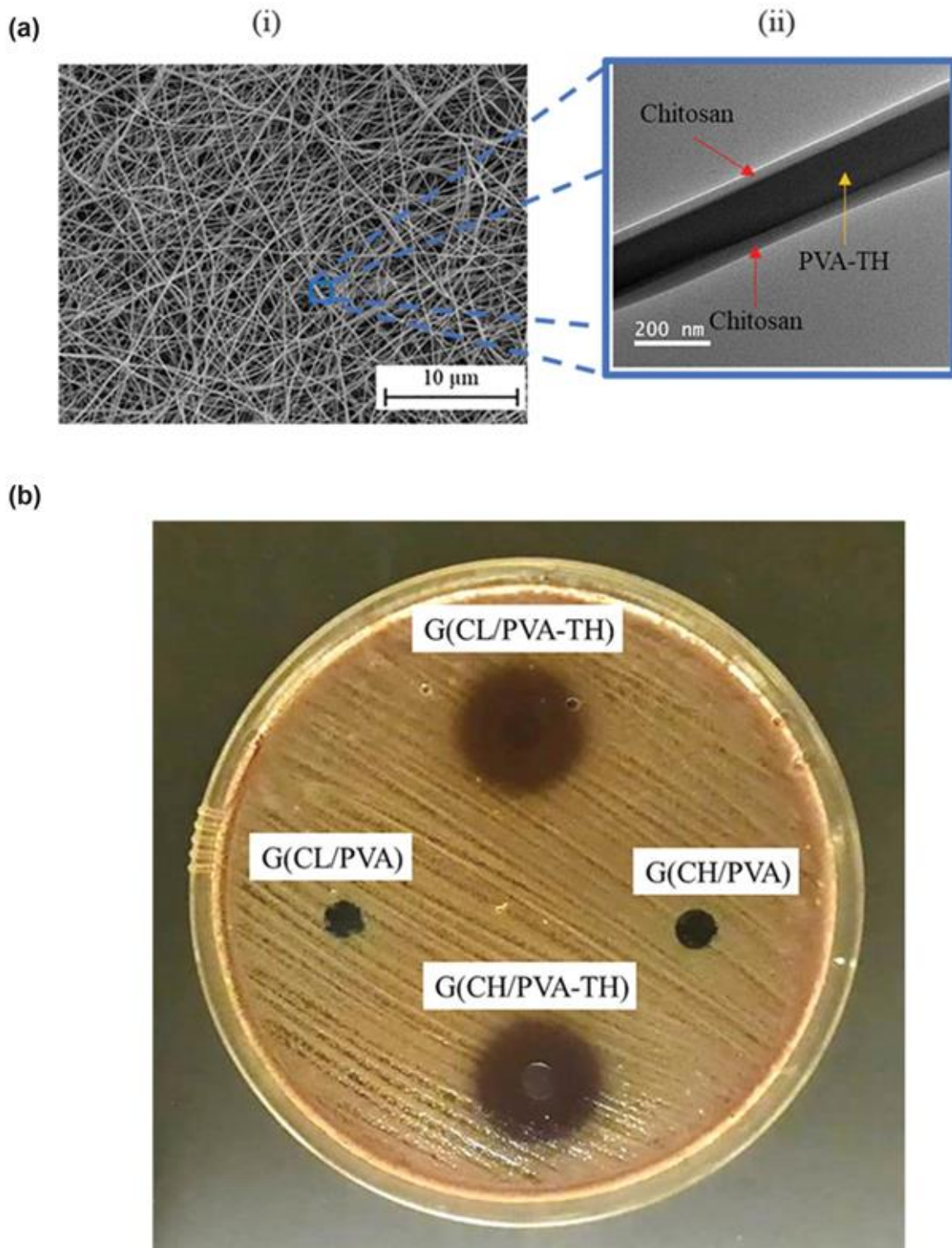


Figure 4.7 (a) SEM (i) and TEM (ii) micrographs of core-sheath nanofibers prepared from chitosan of a low degree of deacetylation (82% (CL)); (b) antibacterial activity of cross-linked nanofiber membranes without TH (G(CL/PVA), G(CH/PVA)) and

containing TH (G(CL/PVA-TH), G(CH/PVA-TH)) toward bacterial strains present in samples extracted from human periodontal subgingival pocket of patients with chronic periodontitis. Reproduced from ref. 8 with permission from Elsevier, Copyright 2020.

In another example, Sattari and collaborators¹⁹⁹ produced composite nanofibers by electrospinning using the coaxial system. Specifically, they produced a chitosan nanofiber shell with gallic acid and grafted oxide graphene cyclodextrin nanofibers with curcumin for a co-delivery system. The results showed that the new formulation presented anti-inflammatory and antimicrobial properties, in addition to being a new approach to the design of synergistic treatments by enabling co-release.¹⁹⁹ In another study,²⁰⁰ chitosan was used together with PVA to produce nanofibers containing gold nanorods (AuNRs) and the anti-cancer agent doxorubicin (DOX). The results showed that the combination of AuNRs and DOX allowed its application as a cell imaging and drug delivery system inhibiting the proliferation and growth of SKOV3 ovarian cancer cells.²⁰⁰

In another study, hyperbranched chitosan was used together with PVA to produce nanofibers containing donepezil with rapid oral disintegration (15 s) for the treatment of Alzheimer's disease.²⁰¹ Nanofibers with an average diameter of 110 to 115 nm showed 97.03% release in a 45 min time interval. In addition, *in vivo* studies using Wistar rats showed that plasma concentration of donepezil was higher when using nanofibers than formulations using thin films.²⁰¹

The blend of chitosan/aloe vera and polycaprolactone was used to produce an antimicrobial dressing based on nanofiber membranes fabricated by sloping free surface electrospinning (SFSE).²⁰² The results showed that the use of SFSE produced nanofibers with higher quality and yield when compared to the electrospinning process employing a single-needle. In addition, the use of aloe vera improved the biocompatibility of nanofibers in human umbilical vein endothelial cells and their antibacterial performance.²⁰² Polyethylene oxide composite nanofibers with cross-linked *N*-maleoyl-functional chitosan were produced with swelling, antibacterial, and triggered antibiotic release properties.²⁰³ The antibiotic tetracycline hydrochloride (TCH) was used as a model for controlled release studies and poly(ethylene glycol) diacrylate and allyl disulfide were used in the UV-irradiation cross-linked treatment. Glutathione was used as a biocompatible

reductant capable of cleaving the cross-linkages with a consequent increase in the release of TCH in the medium.²⁰³

4.2.5.2 *SB-spinning Nanofibers*

Although the first reports of ES processes assisted by air blowing can be traced back to 2005,²⁰⁴ Solution Blow Spinning (SB-Spinning) as an independent technique was first described in 2009 by Medeiros *et al.*²⁰⁵ Similar to ES, the SB-spinning setup is composed by a system of controlled output of polymeric solution, a collector that may be static or rotary and, replacing the high voltage generator, a source of pressurized gas, usually air, that is coupled to the solution nozzle^{180,205–208} (Figure 4.6b). The air flow shears the droplet and at the same time creates a low pressure region in front of the nozzle tip, deforming the droplet in the shape of a cone and then producing capillary jets.^{205,206,208}

This setup allows continuous fiber production with higher yields and at a lower cost when compared to ES, and the simpler components used render the scaling up for industrial applications easier to implement.^{205,207–209} The possibility of spinning without concerns regarding electric conductivity is also responsible for the possibility of fiber deposition directly on virtually any surface, including biological systems.^{205–208} The SB-spinning is not limited by the solute conductivity or the polarizability of the solvent employed, however it is subject to its volatility. The processing of higher volumes of solution coupled with possible irregularities on the air flow account for fibers that usually have a higher mean diameter and wider size distribution than those produced through ES. Thus, both techniques have their advantages and limitations that must be considered for the selected materials. The morphology and diameter of the fibers depend on solution parameters (concentration, viscosity, solvent volatility, and surface tension), processing parameters (nozzle structure, the flow rate of the liquid, gas pressure, and the distance between the tip of the nozzle and the collector), as well as environment parameters (temperature and humidity).^{205,208} Recently, SB-spinning has gained considerable interest in the development of nanofiber drug delivery platforms due to its simplicity, great versatility in terms of material composition, and to the possibility of creating fibers with

different morphologies and structures that allows the encapsulation of diverse drugs as well as a controlled drug release profile.²⁴ Despite the advantages, to date, few studies reported the SB-spinning of chitosan-based nanofibers^{210–215} and none of the prepared structures were applied as drug delivery systems, which open the way for future studies in this field by using this biopolymer.

4.2.5.3 *Physicochemical Characterizations of Chitosan-based Nanofibers*

In general, nonwoven mats have some issues regarding standardized characterizations: the usually random deposition of fibers means a lack of uniformity in density, thickness, and pore size and distribution.²¹⁶ Since most nonwoven mats are based on polymeric fibers, they are usually tested as thin plastics (thickness inferior to 1 mm). Some efforts were made to create appropriate standards to measure properties of textile materials (nonwoven ones included), such as ASTM D1117 (discontinued) and ISO 9073 series.

Nonwoven mats have their morphology typically assessed through Scanning Electron Microscopy (SEM), although optical microscopy sometimes can help to analyze microfibers. Image processing software is used to determine mean fiber diameter and estimate the alignment level. For the mechanical properties, nonwoven mats may follow ASTM D882, regarding thin plastics tests,²¹⁷ although ASTMs D5034 and D5035 are more specifically designed for nonwovens. Typically, the nonwoven mats are analyzed through tensile tests. The wettability properties are covered by ASTM D7334, although the porosity of the material must be considered to avoid false results. Since CS presents hydrophilicity, fibers containing CS in their bulk or surface are expected to present moderate to good interaction with water (contact angle is smaller than 90 degrees), suitable for promoting cellular adhesion and growth.²¹⁸

To effectively determine barrier properties, it is important to verify the permeation to gases and water vapor (ASTMs E96/E96M, D737, and F778), and the analysis of the ability to prevent microbial penetration²¹⁹ is also desired for wound dressing applications. The wound dressings may

also have their adhesion to tissues tested²²⁰ (ASTMs F2255, F2256, and F2258) to determine their ability to keep wounded sites held together. Other biological tests, such as cytotoxicity and microbial growth inhibition, are crucial in determining the wound dressing safety and effectiveness in improving the wound healing process.

The cytotoxicity tests of materials in general are covered by ISO 10993-5 (*in vitro* tests) and ISO 10993-4 (interactions with blood).^{221,222} The cytotoxicity is evaluated by the number of cells exposed to the material that present abnormal characteristics, such as roundish morphology which indicates poor adhesion, altered metabolism, or even cellular lysis or death. The antimicrobial activity is observed as the ability to inhibit the cellular multiplication, either by rendering the cells static, or by effectively promoting cellular death. Solid materials can be analyzed through direct contact, indirect contact mediated by filtering paper or agar, and liquid extracts of the material. For the direct contact tests, it is important that the material presents at least one flat surface. The indirect tests provide a gradient exposure to the material leachate and must ensure the permeating medium will not interfere on the analysis by physically blocking the molecular diffusion or by reacting with the sample. The exposure to liquid extracts may simulate an environment closer to the expected for other regions of the organism that receive the wound dressing. Blood interaction analyses provide valuable data regarding the safety of use as it is desirable that the wound dressing will not cause problems like disturbances in the regular blood clotting process or cause hemolysis. Ideally, wound dressings avoid infections at the same time they present no risks to the host's cells. Organ-on-a-chip templates, although presenting less ethical issues, are still too complex and expensive to be routinely used.²²³

4.3 Summary and Future Perspectives

Chitosan has emerged as a promising biopolymer for the design of novel nanostructured drug delivery systems owing to their appealing properties including biocompatibility, nontoxicity, biodegradability, low immunogenicity, and versatility for chemical modifications. In this chapter, we surveyed recent advances on the use of chitosan for the design of

diverse drug delivery nanocarriers, namely: nanoparticles, micelles, nanocapsules, nanogels, and nanofibers. Such chitosan-based nanocarriers exhibit excellent mucoadhesive and permeation properties, besides providing controlled release of bioactive compounds. Such nano-based platforms have been explored in drug delivery through various routes of administration to improve the treatment of several diseases. Despite the great potential of nanosized chitosan-based drug delivery systems, they have demonstrated not so fast translation to clinical applications, which can be attributed to challenges including the production at a large scale and reproducibility of prepared nanostructures. Additionally, the immunological and toxicological profiles of such nanosystems are not entirely comprehended and further studies are yet required to ensure their safety. In general, it is assumed that the shortcomings will be overcome in the following years, allowing the potential of chitosan-based nanocarriers to be explored in clinical applications.

Abbreviations

AIA	Antigen-induced arthritis
a-OMPC	Anisamide-conjugated <i>N</i> -octyl- <i>N</i> , <i>O</i> -maleoyl- <i>O</i> -phosphoryl chitosan
ASTM	American Society for Testing and Materials
BSA	Bovine serum albumin
CMC	Critical micelle concentration
CS	Chitosan
CsA	Cyclosporin A
CS-NCs	Chitosan nanocapsules
CS-NGs	Chitosan nanogels
CSSAP	Chitosan-stearic
DBA	Benzaldehyde
DMSO	Dimethyl sulfoxide
DOX	Doxorubicin
ES	Electrospinning
HGC	Glycol chitosan
ICG	Indocyanine green

ISO	International Organization for Standardization
LC	L-Carnitine
NCs	Nanocapsules
NGs	Nanogels
NLSC	N-Lysinal- <i>N'</i> -succinyl chitosan
OA	Oleic acid
PM	Polymeric micelles
PMVEMA	Poly(methyl vinyl ether- <i>alt</i> -maleic acid)
pNIPAA	Poly(<i>N</i> -isopropylacrylamide)
PS	Photosensitizers
PTX	Paclitaxel
ROS	Reactive oxygen species
SA	Stearic acid
SB-	Solution blow spinning
Spinning	
SEM	Scanning electron microscopy
TAC	Tacrolimus
TB	Mycobacterium tuberculosis
TPP	Sodium triphosphate
TPPS4	Tetra-phenyl-porphyrin-tetra-sulfonate
HDFn	Neonatal human dermal fibroblast cell line

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Chapter 5

Hyaluronic Acid in Drug Delivery

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5.1 Introduction

Hyaluronic acid (HA), a natural glycosaminoglycan polymer, is composed of glucuronic acid and *N*-acetylglucosamine, which are connected repeatedly by β -1,3 and β -1,4 glycosidic bonds. Its individual disaccharides bind along to form a coiling chain structure of various lengths and molecular weights.¹ HA is the main component of the extracellular matrix and plays an important physiological role in the human body. The wide range of HA molecular weights along with its valuable properties such as

non-immunogenicity, biodegradability, and biocompatibility have resulted in HA becoming more and more used in medicinal approaches specifically in drug delivery systems.² HA and its derivatives can be applied to conjugate with different medicinal molecules such as proteins, peptides, nucleic acids, and various anticancer drugs or as carriers for delivering them to desired site of interests.³ Moreover, HA can specifically bind to cell surface specific receptors, which are widely present in certain tissues and most tumor tissues. This property has paved the way for HA being used in targeted drug delivery systems.⁴ HA-based delivery systems such as nanoparticles, microparticles, and hydrogels, *etc.* can be administrated in different routes that are taken into account in this chapter. In addition, the research progress on different forms of targeted drug delivery systems based on HA are highlighted, including tumor-targeted drug delivery systems. HA's potential for delivery of peptides/proteins and genes are also discussed along with its application in imaging and self-assembling systems which is a novel topic in drug delivery recently. Different applications of this magnificent natural polymer have been summarized (see [Figure 5.1](#)).

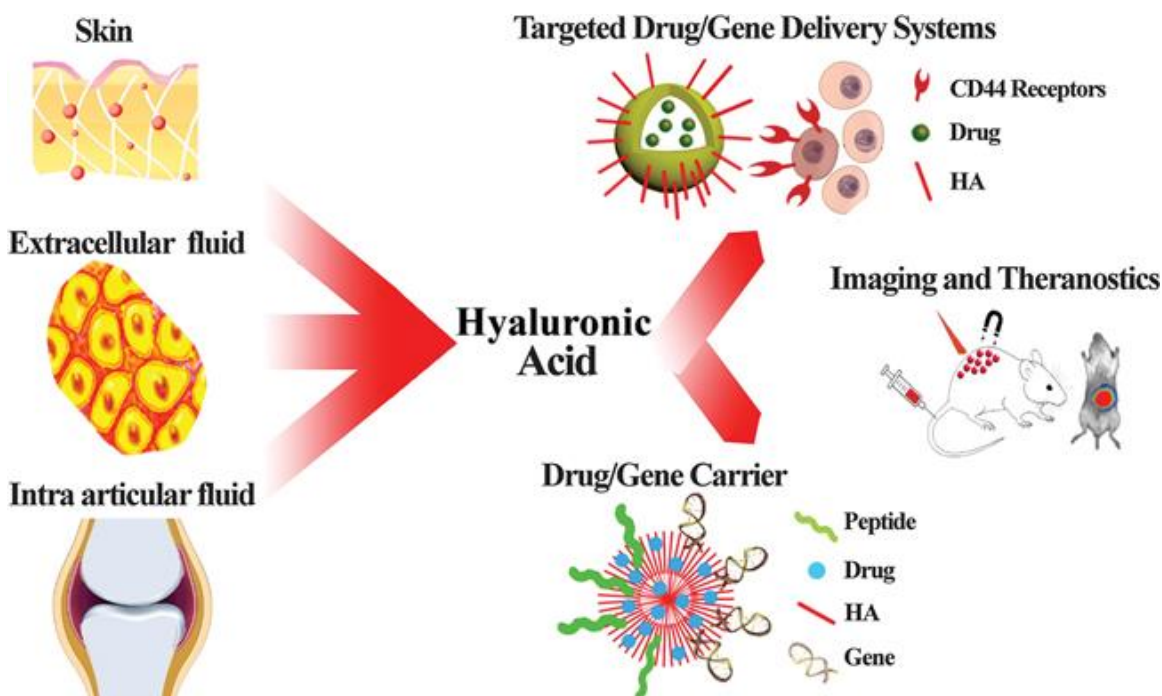


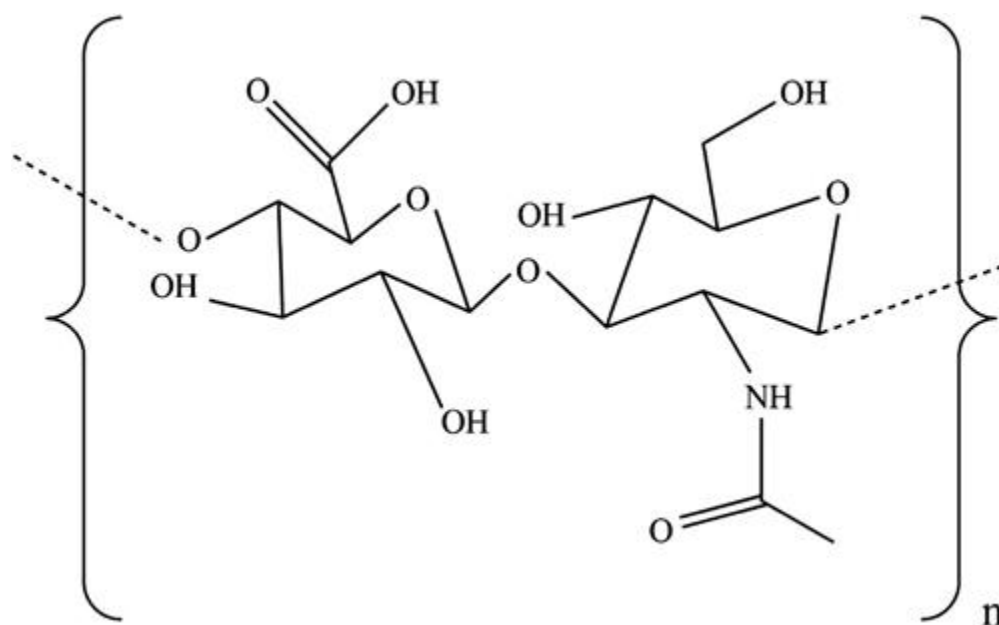
Figure 5.1 Various applications of hyaluronic acid polymer.

5.1.1 Properties

Meyer and Palmer⁵ isolated a high molecular weight glass-like polysaccharide from the bovine vitreous humor. They called it hyaluronic acid. This name is obtained from its glassy appearance and uronic acid. Subsequently, it was separated from the human umbilical cord, bovine aqueous humor, the vitreous humor of pig eyes, groups A and C hemolytic streptococci, human synovial fluid, several mesenchymal tumors, tumors of chicken, and pig skin.⁶ However, it is fair to mention that Levene and Lopez-Suarez⁷ were the first to extract hyaluronic acid with a mixture of sulfated glycosaminoglycans from the vitreous body and cord blood in 1918. They gave the mixture the name 'mucoitin-sulfuric acid.' Isolation of hyaluronic acid from the capsules of groups A and C hemolytic streptococci is of great importance for this biopolymer's industrial production, which Kendall first practiced. Soon after Meyer and Palmer's first publication,⁵ they revealed differentiative features of this new biopolymer from other similar ones. They claimed that the molecular mass of a repeatable unit is approximately 450 Da and pantose is one of the components of hyaluronic acid. It was later proved that 397 Da is the mass of the repeatable disaccharide group, and HA does not contain pantose. The beauties of creation are the transparent appearance of hyaluronic acid aqueous solution, which does not contain any chromophore groups. This property is suitable for filling the vitreous fluid in the eye structure.⁸

Understanding the structural and conformational properties of biopolymers is vital to recognize their functional characteristics. Glycogen, starch, chitin, cellulose, and hyaluronic acid are polysaccharides with different functional roles related to their structure. To exemplify, glycogen and starch are both reserve polysaccharides, which have hydrolyzable alpha-1-4- and alpha-1-6-glycosidic bonds for energy storage. However, chitin and cellulose are structural polysaccharides with rigidity function. The difference in 1-4-glycoside bonds configuration leads to fundamental differences in reserve and the secondary structure and functional properties of structural polysaccharides.⁹ Thus, even from such a brief review, it is clear that biopolymers' physico-chemical and conformational properties play an essential role in their physiological functions.

It is worthwhile examining the functional novelty of hyaluronic acid. Hyaluronic acid (also known as hyaluronan or hyaluronate) is a kind of beta-d-glycan extracellular (or intercellular) polysaccharide. It is a biocompatible linear polysaccharide of the glycosaminoglycan (GAG) family with repeating disaccharide units of $[-\alpha\text{-D-glucuronic acid-}\beta 1,3\text{-N-acetyl-}\alpha\text{-D-glucosamine-}\beta 1,4\text{-}]_n$ ⁸ (see [Scheme 5.1](#)). The most crucial differentiated feature of GAGs from other polysaccharides is their water solubility. While GAGs are water-soluble, other polysaccharides are partially soluble or insoluble in water. Contrary to other extracellular polysaccharides (cellulose and chitin), hyaluronic acid is water-soluble. Members of the GAG family including chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin, keratan sulfate, and hyaluronic acid have five common properties:⁹



Scheme 5.1 Structure of hyaluronic acid.

- (1) GAGs are linear. No branched polysaccharide belongs to this family.
- (2) GAGs are heteropolysaccharides. Repeated disaccharide units are building blocks of GAGs. In contrast, homopolysaccharides are created from identical monomers.

(3) Sulfate groups, such as O-esters or N-sulfates, are the permanent members of GAGs (except for hyaluronic acid).

(4) Acetylation usually occurs on the amino group of the amino sugars; thus, the positive charge often disappears.

(5) All GAGs other than hyaluronic acid are covalently bound to proteins forming proteoglycan aggregates. Not only can hyaluronic acid bind to other components, but it can also be present as a free molecule. In comparison with sulfated GAGs, HA-synthesis does not demand the active synthesis of proteins.

As mentioned above, hyaluronic acid can be distinguished from other members of the GAG family by the absence of sulfate element in its structure. Hyaluronic acid has a broad molecular weight range from 1000 to 10×10^6 Daltons,⁴ and the chemical modification does not occur after its synthesis. The molecular weight of other GAGs is in the range of 4000–50 000 Da.⁹

Hyaluronic acid has lubricating properties because of its ability to absorb a remarkable amount of water.⁹ This property refers to the attraction of osmotically active cations (Na, Mg, K, Ca, and others) to its negatively charged structure in pH around 7. In such conditions, hyaluronic acid adopts an enlarged conformation by binding to a thousand times more water than the weight of the macromolecule itself and forming a gel even at low concentrations. This hyaluronic acid gel media has different histological functions related to its concentration and its molecular weight. The gel's properties such as water-binding, ionic exchange, and size-dependent molecular diffusion, have a functional role in the preventive permeation of large toxin molecules and microorganisms.⁹

Various observed viscosities of hyaluronic acid solutions in the presence of different inorganic salts were surprising for scientists in their early studies. It is an evident phenomenon that the viscosity is related to pH values and the ionic strength of the solution, but it was first elucidated by Fuoss¹⁰ in synthetic polyelectrolyte solutions.

The essential role of hyaluronic acid in joint lubrication is clear to everyone. The *in vitro* studies of Radin *et al.*¹¹ showed the importance of hyaluronic acid lubricative action in the synovial membrane. They measured the friction coefficient of bovine synovial fluid on a motor-driven

glass slider, and it has been shown that hyaluronidase destroyed the lubricating property of this fluid. Swann *et al.*¹² stated that hyaluronic acid's powerful lubricating ability in the synovial fluid could be associated with factors such as length, conformation, state of polysaccharide aggregation, and the protein interaction with HA chains.

Balazs and Laurent¹³ are considered to be the first ones who began the research on the physico-chemical properties of HA. It has been shown that sterilization of HA solution by UV light leads to complete loss of viscosity. Caputo¹⁴ also described that exposure of HA to gamma radiation or electron beams, even at low initial levels of radiation, leads to complete degradation of this molecule.

Hyaluronic acid is a linear non-toxic polysaccharide.⁴ Today, it is known that hyaluronic acid has broad functions such as cell proliferation, migration, differentiation, tissue regeneration, and recognition at all stages of organism development. Hyaluronic acid's biological activity is highly size-dependent and depends on its molecular weight (M_w), making HA fragments superior regulatory signal molecules.⁸ A progressive research field in medicine will be the isolation of oligosaccharide fragments of HA to investigate its functional properties.⁹ HA molecules with 500 000 Da and higher molecular weights can inhibit cell proliferation and migration, whereas smaller ones (50 000–100 000 Da) act quite conversely and stimulate cell proliferation. For instance, hyaluronic acid with a molecular weight of 100 000 Da stimulates fibroblast growth in the collagen matrix. The growth inhibition effect of high molecular weight hyaluronic acid (6 000 000 Da) in synovial fluid is another notable example. Thus, hyaluronic acid shows mutually opposite features based on its molecular weight. Other functions of HA with high molecular weight (more than 500 000 Da) are inhibition of blood and lymphatic capillaries growth, prevention of cartilage breakdown, and reductive effect on inflammatory processes. However, hyaluronic acid's growth stimulation with 50 000–100 000 Da has a vital role in wound healing and migration of cells into the wound site. This contradictory action of hyaluronic acid also can be seen in its smaller fractions. As an illustration, hyaluronic acid with 3–10 disaccharide units (1300–4500 Da) instigates endothelial cell proliferation. By contrast, the 1300–7200 Da (3–16 disaccharide units) fractions while

inhibiting the proliferation of epithelial cells, do not have a suppressive effect on the proliferation of fibroblasts and smooth muscle cells. Thus, hyaluronic acid with molecular weights less than 30 000 Da can penetrate the trans-epidermal barrier. And what's more, is the improvement of microcirculation and nourishment of the tissue with the aim of capillaries growth stimulation by these small HA fragments (less than 30 000 Da). Medium-size fragments of HA (25–50-disaccharides long) particularly accelerate inflammation and they are immuno-stimulants with powerful angiogenic activities.⁹

Hyaluronic acid signaling systems such as cluster determinant 44 (CD44), RHAMM (receptor for HA-mediated motility), TLR4, HA receptor for endocytosis (HARE),^{15,16} lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1), IVd4, and LEC¹⁷ are the well-known receptors for hyaluronic acid. The B(X7) B sequence is similar to CD44 and RHAMM, which will bind to HA. B is an essential amino acid residue, and the X's contain at least one essential amino acid. HA–CD44 signaling affects Rho and Rac1 GTPases. This interaction leads to reorganization of the actin cytoskeleton ErbB2-driven cell motility and cell proliferation through src-related tyrosine kinases and NF-κB. Interaction of high molecular weight HA with CD44 results in anti-inflammatory properties by blocking cytokine signaling, whereas the low molecular weight HA fragmentation products of HA interact with TLR4 (toll-like receptor 4) and consequently cause cell differentiation and inflammation by phosphorylation of p38/p42/p44 MAP-kinases and NF-κB. The result of HA:RHAMM interaction is cell movement, which is activated through a tyrosine kinase signal transduction pathway (pp60c-src) targeting focal adhesions. Thus, HA has a pivotal role in morphogenesis.¹⁸

5.1.2 Synthesis and Degradation

As mentioned in the properties section, hyaluronic acid has a simple disaccharide structure. After the synthesis of hyaluronic acid, no chemical modification happens. The method of hyaluronic acid synthesis is similar to the way that cellulose chains are synthesized in plant cells. Hyaluronan synthase (HAS) is a membrane-bound enzyme, which produces hyaluronic acid at the cell surface and extrudes it through the membrane into the

extracellular matrix during chain elongation. In contrast, the Golgi apparatus is the production site of other glycosaminoglycans.⁹ As mentioned above, hyaluronic acid is excreted into the cellular matrix after its synthesis. Thus, the endocytosis mechanism is essential for the transportation of HA into the cell nucleus.⁹

HAS enzymes are membrane proteins that are located in the cell membrane. To better understand which enzyme has the pivotal role in embryonic evolution, intentional mutations on the HAS gene were done in mice experimental groups. It turned out that mutation in HAS1 and HAS3 genes resulted in viable mice with deficiencies, whereas mice with a mutated HAS2 gene had severe deficiencies. Thus, a mutation in the HAS2 gene during embryonic development is lethal.¹⁹ High molecular weight HA is the product of HAS1. HA with a molecular weight of up to 2 000 000 Da is the result of HAS2. Short HA chains (200 000–300 000 Da) are produced from HAS3. The activity of HAS enzymes is as follows: HAS1 < HAS2 < HAS3. Therefore, HAS3 is the most active one.^{20,21}

The activation of these three genes of HAS has a pivotal role in the cell cycle. For instance, HAS-2 is activated highly through the transformation step of the differentiated cells. In contrast, HAS-3 is suppressed during transformation. This conclusion was made by Yung *et al.*²² with the PCR method's aim, which described the corresponding modifications in mRNA expressed from HAS-1, HAS-2, and HAS-3 genes in an *in vitro* model of peritoneal mesothelial cell damage. It was found that the expression of mRNA for the HAS-1 enzyme, which is responsible for the production of high molecular weight HA, is down-regulated through the repair phase of the cells. However, HAS2 and HAS3 genes are up-regulated in this phase.

Degradation of HA can take place in the lymph node, liver, spleen, and kidneys. The rate of synthesis and degradation of hyaluronic acid is different in various tissues. For instance, the synthesis rate and decomposition rate of HA in the skin are similar, and it has a 24–48 h half-life. In contrast, mature collagen has a half-life of several months in skin tissue.⁹ The regulation mechanism of HA synthesis is created by phosphorylation–dephosphorylation of HAS. Several phosphorylation sites in HAS exists, which are phosphorylated through protein-kinase C&A. Thus, the activators of protein-kinase C&A are stimulators of HA

synthesis.⁹ Degradation of HA in tissues takes place first by lymphatic tissue; then the degraded HA enters the blood to find its way through hepatic cells, where it is metabolized to glucuronic acid and *N*-acetylglucosamine in lysosomes. The entrance of HA to hepatic cells takes less than a minute *via* receptor-mediated endocytosis. CO₂, H₂O, and urea are the final products of HA metabolism. Rapid physiologic turnover of HA is so that HA has a half-life of 2.5–5.5 min. Therefore, the expected concentration of HA in the blood is low except for abnormal conditions such as inflammatory response, immune response, and tumorigenesis.⁹

Destruction of hyaluronic acid is possible through enzymatic and non-enzymatic reactions.⁹ Non-enzymatic reactions, which hyaluronic acid is sensitive to, are acid–alkaline hydrolysis, oxidation–reduction degradation with phosphate ions, radiolysis, heat degradation, and ultrasonic depolymerization.²³ The irreversible decrease of HA's viscosity is the result of its acidification.¹³ Complete hydrolyzation of HA into glucuronic acid, glucosamine, acetic acid, and carbon dioxide will occur by HA reaction with mineral acids. The reaction of diluted sulfuric acid with HA leads to the formation of disaccharide crystals.²⁴ Oxidation–reduction is another non-enzymatic depolymerization of HA. Swann²⁵ observed the depolymerization of HA at pH 3 by ascorbic acid. Isolation of HA in atmospheric conditions may lead to the destruction of HA. For instance, it was observed that the viscosity of HA prepared in a nitrogen-purged space was more significant than the HA prepared in air. This oxidation–reduction is catalyzed by heavy metals. For instance, the application of 8-hydroxyquinoline led to inhibition of the spontaneous depolymerization of HA in its purification process from proteins by trypsin containing Fe³⁺ ions.²⁶ Thus, the clearance of HA-contained samples from heavy metal sources (blood) is essential for the maintenance of the polymerization level of HA.⁹ Furthermore, degradation of HA solutions during lyophilization was observed in the presence of phosphate ions.²³ Sterilization of HA solutions in an autoclave at 120–130 °C or by ionizing gamma radiation results in significant depolymerization of HA. In order to protect the HA solutions against depolymerization, the addition of amino acids, boric acid, glycerol, hydroxyproline sulfate, uric acid, and phenolic compounds (pyrogallol) is suggested.⁹ For efficient sterilization of HA solutions by

gamma radiation, the usage of depolymerization inhibitors like copper sulfate and sodium ascorbate was described by²⁷ I. I. Samoylenko and L. I. Stekolnikov (RF Patent No. 2051154, C 08 V 37/08). In this sterilization process, one gram HA was dissolved in 100 ml 0.9% NaCl solution. Then, the solution was purged with argon for 30 min at 10 ml min⁻¹. The addition of polymerization inhibitors is the next stage (0.02–0.04 g of copper sulfate and 0.06–0.08 g of sodium ascorbate).

Enzymatic depolymerization of hyaluronic acid occurs by the action of the hyaluronidase.⁶ The first sparks of hyaluronidase discovery date back to 1928, when Duran-Reynals injected subcutaneously the Duran-Reynals spreading factor, the biological material that was obtained from rabbit testicles and contained hyaluronidase with black ink. The fast distribution of black color was observed. Furthermore, it turned out that increased permeability of connective tissue was the result of hyaluronic acid depolymerization.²⁸

In the family of human hyaluronidase, at least seven types have been found. Meyer²⁹ divided the hyaluronidase into three types: testicular-type hyaluronidases, hyaluronate-*endo*- β -glucuronidase, and hyaluronate lyase. This classification is based on the enzyme's source, the final product of the enzyme, and the catalytic reaction. Hyaluronidases of the testicular type (Type 1) is called hyaluronate-*endo*- β -*N*-acetyl hexosaminidase and have three subtypes. Type 1a (testicular hyaluronidase) is present in the animal seminal glands and sperms. Type 1b (lysosomal hyaluronidase) is contained in the lysosomes, blood serum, and synovial liquid. Type 1c (submandibular hyaluronidase) is present in saliva and saliva glands. Hydrolysis by Type 1 and Type 2 results in the formation of tetrasaccharides, which possess amino sugars at their reducing terminus. Furthermore, transglycosylation is another ability of testicular and lysosomal hyaluronidases. As a result, a transition of disaccharide fragments between the molecules is possible. The activity of Type 1 hyaluronidase is pH-relative. The testicular type is active in the pH range of 4–7, while the lysosomal and submandibular types of activation are in the pH range of 3.5–4.5. Deactivation of these enzymes takes place at pH 8.2. Type 3 hydrolyzes β -*N*-acetylaminoglycoside bonds and dehydrates the residue of uronic acid.⁹

Numerous factors control the activity of hyaluronidase. For instance, hormones have mutual controlling roles on hyaluronidase activity. While adrenocorticotrophic hormone (ACTH) and cortisone suppress the activity of hyaluronidase, thyroid and somatotropin are activators. Histamine, serotonin, cysteine, and protamine are other activators. Proteins (glycoproteins, plasma albumins, lipoproteins, pepsin, trypsin) and polysaccharides (high concentration heparin, chondroitin sulfate) are inhibitors. Other well-known inhibitors are salicylates, magnesium salts, cuprum oxide, iodine, hydrogen peroxide, cyanides, and sulfamides. Hyaluronidase inhibitors are a helpful tool for reducing HA depolymerization during its extraction.⁹

The equilibrium rate of synthesis and degradation of substances in the human body is essential to maintain homeostasis. Thus, hyaluronic acid, as a biopolymer, is not an exception. The maintenance of HA's homeostasis is possible through receptor-mediated degradation at LYVE-1.²⁰

Unfavorable by-products of hyaluronic acid, which have detrimental biological effects, are of great concern in drug delivery systems. A striking illustration of the undesirable effect of hyaluronic acid degradation products is the neovascularization and pro-inflammatory response of low molecular weight hyaluronic acids, which are degraded from high molecular ones. Besides the wound-healing role of smaller HAs (hyaluronic acids), tumor progression and metastasis are their inevitable effects. Thus, it is worthwhile alleviating the adverse effects through optimization of the degradation profile and observation of cellular responses.¹⁸

5.2 Application of Hyaluronic Acid in Drug Delivery Systems

Hyaluronic acid displays practical applications in drug delivery, since it has some characteristics such as biocompatibility, biodegradability, simplicity in chemical modification, high ability in drug loading, and having inherent targeting properties thanks to precise interactions with receptors like CD44, receptor for hyaluronate-mediated motility, glial HA binding protein, and LYVE-1, among which CD44 is expressed on various cells.^{30–32} Because of these properties, HA can be chemically linked to drugs or drug carriers,

bringing some benefits including addressing solving issues, rising the plasma half-life of a drug resulting in reducing the speed of clearance, strong probability of tumor targeting, enhanced drug distribution in tumor tissues, and developed drug selectivity in target cells specially in cancer stem cells.³³ Such advantages have attracted scientists' attention to use HA in distinctive drug delivery systems which are described in the following sections.

5.2.1 HA in Parenteral Delivery

Conventional drug delivery systems, in which poly lactic glycolic acid (PLGA) is used, showed inflammation and protein denaturation resulting from the degradation of PLGA, whereas HA emerged as a protein-friendly drug carrier, resulting in being practical in sustained release (SR) formulations of protein and peptide drugs by parenteral delivery,³⁴ and the first use was in 1991.³⁵

First, microparticles of HA for SR of human growth hormone were developed through a solvent evaporation method and spray dry technique. The first approach resulted in the generation of large particle size, the consequence of which was low patient compliance; nevertheless, the second approach resulted in the production of small particle size; subsequently, the patient compliance was enhanced, yet this method was hard to scale up.³⁶ In another study, nanoparticles of HA-PEG-PLGA copolymers were synthesized for delivering 5-fluorouracil (5-FU) to the tumor cells. The *in vivo* tests were pursued following intravenous administration of HA-PEG-PLGA-FU nanoparticles indicating the safety, biocompatibility, and hemocompatibility of nanoparticles for intravenous administration.³⁷ Meiling *et al.*³⁸ explored the effect of HA in drug delivery through developing a targeted system where glucocorticoid prednisolone was encapsulated in solid lipid nanoparticles coated with HA for treating rheumatoid arthritis. After intravenous injection into mice, it was revealed that such a targeted system was maintained for a more prolonged period in blood circulation in comparison with a system without HA. The impact of HA in the treatment of rheumatoid arthritis through parenteral delivery is also indicated in other studies.³⁹

Added to these, for parenteral drug delivery, some scientists fabricate a hydrogel of HA, consisting of hydrophilic polymers connected to each other either through covalent bonds or physical interactions.⁴⁰ In this regard, Fang *et al.*⁴¹ generated temperature-sensitive hydrogels composed of poly(*N*-isopropylacrylamide), poly(*N*-isopropylacrylamide) with chitosan, and poly(*N*-isopropylacrylamide) with chitosan and hyaluronic acid. The gelation temperatures of the hydrogels were below body temperature, causing them to become a gel quickly and easily, which make them appropriate candidates for injectable drugs. HA hydrogels did not disintegrate in *in vitro* and *in vivo* environments, because HA can prevent such disintegration; furthermore, HA caused the lower usage of poly(*N*-isopropylacrylamide), making profound contributions for decreasing toxicity. In another study, the effect of HA in parenteral drug delivery was explored, in which it was coated non-covalently, resulting in enhancing the cellular uptake of liposomes by MG63 cells through CD44 receptors.⁴²

5.2.2 HA in Pulmonary Delivery

Since numerous parenteral peptide and protein drugs may become undesirable, pulmonary drug delivery of such drugs has been investigated.⁴³ For developing such systems, HA can be suitable, since it has high stability in lungs.⁴⁴ In this regard, HA was adopted to an insulin solution, the consequence of which was an enhancement in the absorption following the intratracheal administration to rats.⁴⁵ Similarly, dry powder of HA and insulin were prepared through a spray dry method for inhalation administration. The results of this study revealed the significant impact of HA in extending the mean residence time and terminal half-life.⁴⁶ In another study, Xie *et al.*⁴⁷ synthesized a HA–cisplatin conjugate, followed by testing both the intravenous infusion and pulmonary route. The results of *in vitro* tests indicated that pulmonary drug delivery had higher cisplatin accumulations in the lung tissue and a sustained release plasma profile, yet *in vivo* tests should be conducted for obtaining more precise outcomes. Also, the suitability of HA was investigated by Li *et al.*⁴⁸ fabricating inhalable microparticles of HA and salbutamol sulfate with *in vitro* release continued for 20 hours which was longer than pure salbutamol sulfate. Additionally, the potential application in pulmonary drug delivery of the

microspheres of HA and alginate were explored, demonstrating their feasibility for such application as they had low density ($0.035\text{--}0.063\text{ g m}^{-3}$), high porosity ($97.6\text{--}98.7\%$), and high BET-specific surface areas (446 ± 76 and $611 \pm 49\text{ m}^2\text{ g}^{-1}$).⁴⁹

5.2.3 HA in Ocular Delivery

HA can increase the ocular residence time due to its viscosity and pseudoplastic behavior which would contribute to its mucoadhesive function. What is more, its characteristics, such as being non-irritating and having a high water-binding capacity, highlights its applications in ocular drug delivery systems,^{31,34,43} where it can be adopted as a carrier or surface modification material, drug–polymer conjugate, and carrier matrix to make viscose solutions, hydrogels, and implanted stents.⁵⁰

Ophthalmic viscose solutions containing HA are suitable for topical administrations thanks to their pseudoplastic behavior, resulting in dropping and blinking without running out of the eye.^{51–53} Moreover, HA is absorbed by CD44 which are on the surface of the corneal epithelial cells, resulting in its adhering to the surface of the eye.⁵⁴ Since such solutions have a limited retention time despite their several benefits, some scientists developed hydrogels of HA for ocular drug delivery, which bring consequences like improving biocompatibility, increasing sustained drug release, and extending the retention time.^{55–58} Additionally, nano-carriers of HA have been developed, and such carriers showed promising outcomes including providing high transfection, increasing drug residence time, and complete release of the drug,^{53,59–61} whereas Contreras-Ruiz *et al.* revealed that the distribution of nanoparticles of HA/chitosan was different in human corneal epithelial cells.⁶² In comparison with viscose solutions, solid dosage forms manifest a more-gentle-sustained drug release as the polymer would have more water swelling steps prior to drug elution.⁵⁰ Subsequently, scientists developed various forms of solid dosage, among which inserts and implanted stents were widely employed for ocular solid preparations. Ophthalmic inserts provide a long corneal contact time and suitable bioavailability. Using HA in such inserts makes the corneal contact time adequately long, as it significantly delays the ability of HA chains to

disintegrate, followed by slowing down the diffusion rate of the drug from the swelling matrix.^{63,64} Moreover, implanted stents with HA bring benefits like rising cell viability and promoting a neuronal phenotype.⁶⁵

5.2.4 HA in Nasal Delivery

Although nasal drug delivery has significant advantages such as rapid absorption, it has a main challenge, poor bioavailability. One method for solving this challenge is improving the absorption of drugs and proteins through mucosal tissues by employing polymers with a high bio adhesion ability like HA.³¹ In a previous study, it was indicated that the nasal absorption of protein drugs can be improved by using HA modified with tetraglycine-l-octaarginine, since a five-fold rise in the bioavailability of somatropin was revealed.⁶⁶ In another study, HA was adopted to enhance the uptake of the alloy–drug nano-conjugates which were applied for the treatment of glioblastoma.⁶⁷ Also, the treatment of chronic rhinosinusitis with nasal polyposis was explored by applying HA in sprays and nasal drops, indicating a promising enhancement in the patients' signs.⁶⁸ Furthermore, it is demonstrated that chronic bronchitis can be treated by nasal HA accompanying a prescribed antibiotic, which would require a higher dose of antibiotic in the case of not using nasal HA.^{69,70} Additionally, Lim *et al.*⁷¹ revealed that HA/chitosan microspheres may have suitable bioavailability upon nasal administration of gentamicin compared to using pure chitosan and HA.

5.2.5 HA in Oral Delivery

To explore the impact of HA in oral drug delivery, polymeric niosomes containing HA were fabricated for the oral delivery of quercetin. The results of this study indicated the suitable potential of such niosomes for this drug delivery system.⁷² In another study, nano emulsions of miconazole nitrate and HA were fabricated, indicating the adequate pseudoplastic flow of the formulation, resulting in its potential for oral drug delivery.⁷³

5.2.6 HA in Vaginal Delivery

In a study of vaginal drug delivery, mucoadhesive polymers including HA, which was thiolated and preactivated with 6-mercaptopnicotinamide were formed. The consequences highlighted the potential of preactivated HA in vaginal drug delivery, since they had 3.6-fold prolonged disintegration time in comparison with unmodified HA.⁷⁴ What is more, HA-based films were generated for vaginal delivery of metronidazole. The outcomes of this study revealed that such films would persist in the vaginal environment for a longer duration and may have slow drug release (at least 6 days).⁷⁵

5.2.7 HA in Topical Delivery

The extracellular matrix (ECM) of most vertebrate connective tissues has a considerable amount of HA, 50% or more of which is the HA existing in skin.⁷⁶ This causes the wide range usage of HA in topical delivery. In a recent study, Kleinubing *et al.*⁷⁷ showed that nano emulsions consisting of HA can be suitable in delivery systems of lipophilic drugs, as the physicochemical properties and physical stability of topical nanostructured systems containing *P. pubescens* fruit oil were enhanced by employing such nano emulsions. In another study, hybrid nanogels of ovalbumin-conjugated hyaluronic acid–methacrylate and schizophyllan–methacrylate were fabricated to explore their potential for topical drug delivery. The consequences of this study indicated an enhancement in the efficacy of ovalbumin delivery.⁷⁸ Added to these, nanoparticles of HA and chitosan were generated in order to evaluate the delivery of clindamycin to pilosebaceous units, in which the nanoparticles with HA were more significantly diffused, yet both the nanoparticles had suitable impacts on the enhancement of targeted delivery of clindamycin.⁷⁹ Also, Yang *et al.*⁸⁰ synthesized a type of bionic ECM hydrogels based on thiol-modified poly and oxidized hyaluronic acid, which were suitable for initial wound coverage as well as extending the life span of the dressing thanks to their adaptability and self-healing potential, which were due to the aldehyde groups in the oxidized hyaluronic acid. Additionally, nanofibers of HA/polyvinyl alcohol/polyethylene oxide blend embedding a new ZnO nanoparticle/cinnamon essential oil were developed, which demonstrated the effects of such nanofibers on impeding the growth of *Staphylococcus aureus* and highlighted their positive effect on wound healing.⁸¹ Similarly,

fibers of poly(ϵ -caprolactone)/polyethylene oxide loaded with HA and keratin were developed in a study whereby their effectiveness for wound healing purposes was indicated.⁸² Identically, the noteworthy influences of the fibers of HA in wound healing were investigated in other studies,^{83–86} which reveal the suitability of such fibers, mainly thanks to the biocompatibility, biodegradability, viscoelasticity, and non-immunogenicity.

5.2.8 HA in Tissue and Regenerative Medicine

Tissue engineering has become an emerging field for regenerating or repairing tissues. One of the crucial factors in this science is the biomaterial scaffold, which should be biocompatible and frequently present in the ECM of tissues. Considering this, HA is an appropriate candidate in tissue and regenerative medicine.^{31,87} In a study, cryogels based on an interpenetrating polymer network of gelatin methacrylate and HA were formed for soft tissue engineering targets. The results of this study demonstrated that increasing the concentration of HA may improve the mechanical properties of cryogels while limiting the reversibility characteristics, yet the generated cryogels with all amounts of HA had the appropriate strength and Young's modulus (G-H-20, $E = 6$ kPa).⁸⁸ Also, Li *et al.*⁸⁹ highlighted the effects of a biomimetic hyaluronic acid oligosaccharide-based composite scaffold for bone tissue engineering. They showed that this kind of scaffold would be beneficial for such purposes, since it may enhance the attachment of endothelial cells, facilitate the osteogenic differentiation of MC3T3-E1 and BMSCs, and have an ideal biocompatibility and tissue regenerative capacity. Additionally, the role of HA in salivary gland tissue engineering was explored by Lee *et al.*,⁹⁰ who demonstrated that the generation of salivary gland organ germ would be promoted by adopting high molecular weight HA. Moreover, injectable composite hydrogels of HA–tyramine with regenerated silk-fibroin solutions have been investigated for cartilage tissue engineering, revealing that such hydrogels were biocompatible and would enhance the expression of cartilage matrix proteins giving rise to ECM production by chondrocytes.⁹¹ Identically, an injectable, click-crosslinking HA hydrogel scaffold modified with a bone morphogenetic protein-2 mimetic peptide was fabricated for bone tissue engineering. This scaffold significantly prolonged the availability of bone morphogenetic

protein-2 mimetic peptide, making contributions to its applications in bone tissue engineering.⁹² In another study, for brain tissue engineering, polymeric scaffolds containing HA, collagen, and chondroitin sulfate were formed, and the results highlighted the suitability of such scaffolds for enhancing neurogenesis, meaning that they would be practical in brain tissue engineering.⁹³ In addition, Movahedi *et al.*⁹⁴ developed polyurethane/starch/HA nanofibers for skin tissue engineering purposes and showed the appropriate role of such nanofibers in cell promoting as well as cell attachment.

5.2.9 HA in Anti-cancer Drug Delivery Systems

The first cause of death in developed countries and the second one among developing countries is cancer. This disease begins with an unusual proliferation of body cells. If the messenger RNA, which is sent by genes to cells, is unclear, cell function will be abnormal. Therefore, abnormal cells circulating in the blood or immune system can migrate and induce tumors. Cancerous cells are able to spread into different parts of the body and obstruct healthy cells to gain oxygen. Conventional ways to treat cancer, such as chemotherapy and radiotherapy, have harmful side effects on normal cells. Other disadvantages of these treatments are the low solubility and blood circulation time of the drugs.⁹⁵ Therefore, it seems necessary to design new therapeutic methods. Drug delivery systems, based on nanotechnology by overcoming the cell barriers, not only carry anticancer-drugs to tumor sites with less harm to other organs, but also have a great capacity to load photosensitizers, photothermal agents, immune regulators or stimulators, and radiation sensitizers, making combined therapeutic methods possible and efficient.^{96,97} In addition, anti-cancer agents in subcutaneous formulations, like peptide and proteins, have low solubility, and their toxicity makes the treatment efficiency poor.³⁴ This issue can be solved with the application of nano-carriers. Biocompatibility and biodegradability of natural polymers have exhibited them as excellent candidates to be used as drug carriers.^{33,98} A non-toxic and non-immunogenic natural polymer is hyaluronic acid which, as a linear polysaccharide, could be modified easily to be used for different applications in cancer therapy.⁹⁹ While some polymeric carriers, such as

polyethylene glycol, are not biodegradable and do not have a noticeable payload, HA biocompatible and biodegradable nanoparticles can solve these limitations by presenting a high potential for loading drugs as carriers and targeting agents, simultaneously, due to their interaction with overexpressed CD44 receptors on significant types of solid cancers.³⁰ Also, hyaluronic acid has a great capacity chemically to be linked to other carriers or drugs. First, as colloidal carriers, HA's associations to micelles would improve solubility problems without changing the micelle function.³³ Furthermore, by decorating the carriers' surface with HA, the composite could target the tumor cells because of their affinity for specific receptors, including CD44.³¹ Secondly, anti-cancer drugs with a low molecular weight usually have a short *in vivo* half-life and indiscriminate body distribution, causing numerous side effects.¹⁰⁰ To overcome the problem of cell biodistribution pattern, HA can actively target cancerous sites to improve the drug distribution in tumors.^{101,102} Additionally, loading the drug to HA would enhance stability in a biological environment and extend the time of circulating the nanoparticles in the blood.¹⁰³ Therefore, the enhanced permeability and retention (EPR) effect leads the drug to diffuse through the leaky tumor vasculature. Consequently, the accumulation in the tumor would be increased, called passive targeting.^{4,104–106}

Considering resistance against chemotherapeutic drugs in several patients, no tumor response is seen unfortunately.¹⁰⁷ Multidrug resistance (MDR) causes cross-resistance to a broad spectrum of agents which have not been exposed to the cell, being induced by exposure to one drug.¹⁰⁸ Another merit of using hyaluronic acid modification in cancer treatment is overcoming the MDR effect. CD44, a cell-surface glycoprotein is a viable therapeutic target overexpressed in many solid tumors and drug-resistant cancer cells. As it has an essential role in cell interactions, adhesion, and migration, targeting CD44 and delivering a therapeutic agent simultaneously may overcome MDR and improve patient prognosis.¹⁰⁹ Also, HA could benefit the delivery of cytotoxic agents by restricting general absorption *via* inhibiting the drug from penetrating into the outer skin barrier and inserting within the stratum.¹¹⁰ HA has high stability and can bypass the complement activation system. It is noteworthy that all the above-mentioned advantages depend on the molecular weight of HA. Liver

and kidneys eliminate 1200 kDa HA; thus, it has low transfection efficacy compared to HA molecules with low molecular weights up to 150 kDa.¹¹¹ We have summarized some examples regarding the application of HA in cancer drug delivery systems (see [Table 5.1](#)).

Table 5.1 HA in anti-cancer drug delivery systems.

Anti-cancer delivery system	Drug/gene loaded	Target cells	Results	Ref.
Conjugated HA with a fluorescent probe	Mitomycin C or epirubicin	Metastatic lung carcinoma model, Lewis lung carcinoma cells, and MH-134 tumor	Number of metastatic lung nodules was decreased.	112
HA–Drug bioconjugate micelles	Quercetin	H22 tumor-bearing mice and MCF-7 cells	Cytotoxicity was increased four times.	113
HA–cystamine–poly(lactic-co-glycolic) nanoparticles	Doxorubicin and cyclophosphamide	Breast cancer stem cells	A synergistic anti-tumor effect with a lower survival rate of tumors than the monotherapy method was observed.	114
HA decorated nanocomposites (Laponite [®] nanodisks)	Doxorubicin	HeLa cells	Cytotoxicity of nanocomposites was increased.	115
HA–antibody conjugated gold nanorods	Photosensitizer 5-aminolevulinic acid	MCF-7	Significant improvement in the targeting	116

	c acid and imaging agent Cy7.5 for photodynamic therapy		and cytotoxicity by improved internalization.	
Cholanic acid- derivatized HA biopolymer wrapped around a single-walled carbon nanotube	Doxorubicin	OVCAR8/ADR drug-resistant xenograft model	More effective in killing drug- resistant cancer cells – complete tumor eradication was observed.	109
HA–cleavable peptide–Au nanoparticles	Docetaxel	HeLa tumor- bearing mice	Enhanced cytotoxicity and anti-tumor efficacy.	117
Functionalized mesoporous silica nanoparticles with biotin- modified HA	Doxorubicin	Colon cancer cells	Apoptosis efficiency was increased.	118
Conjugates of tLyP-1-PEG- TOS (tLPTS) and TOS- grafted HA	Docetaxel	PC-3 and MDA-MB- 231 cells	High drug encapsulation efficiency (93%), sustained drug release behavior, enhanced intracellular delivery and increased anti- invasion ability,	119

			cytotoxicity, and apoptosis was exhibited.	
HA nanogels encapsulated for gene silencing	Green fluorescence protein siRNA	HCT-116 cells, which have CD44 receptors on their surface.	Nanoparticles were taken up selectively.	120
HA–chitosan nanoparticles	miR-34a with doxorubicin	Breast cancer	The expression of non-pump resistance and anti-apoptosis proto-oncogene Bcl-2 was reduced and miR-34a inhibited breast cancer cell migration.	121

5.2.9.1 Drug-conjugated HA Systems

Although many free drugs show effective anti-cancer performances against tumor cells, they are poorly soluble in aqueous medium and suffer low bioavailability.¹²² In order to solve these dilemmas, it is highly recommended to conjugate the anti-cancer agents to a suitable polymer, creating a systemically non-toxic covalent bond.⁹⁸ To improve the stability of the drug, the linker should be stable in circulation. As a result, the polymer would be able to prevent the deactivation of the drug during circulation.¹⁰¹ Due to these benefits, drug conjugation is also known as “prodrug”. The point that makes researchers focus on prodrugs is their ability to control drug release and active targeting through a selective CD44 receptor-mediated process, localizing the drug performance in cancer. Internalizing into the cancer cell, a hydrolytically labile conjugation bond would be hydrolyzed by intracellular enzymes inside the tumor, and regenerate the cytotoxic agent to release the drug.¹²³ For the first time, in

1996, Akima *et al.* reported HA as a suitable polymer to be conjugated with Epirubicin because of carboxylic groups, presenting a wide range of biomedical applications of hyaluronic acid.¹¹²

For chemical modification, the sites being provided by carboxylic groups are appropriate to be conjugated with drugs, which exist in the molecular structure of HA. Carboxylic groups of HA can participate in amidation and esterification reactions. The acetyl groups in the HA structure are also a potential site for drug conjugation.¹²⁴ The improvement of therapeutic efficacy of the prodrugs in comparison with the free drugs depends on the degree of substitution of HA with the anti-cancer drugs.⁴

Sodium butyrate, cisplatin, doxorubicin, paclitaxel, docetaxel, camptothecin, and mitomycin C are among the drugs that could be conjugated with HA.^{125–131} In these composites, HA plays the role of carrier and targeting moiety for CD44 receptors.³³ Since most cytotoxic drugs are hydrophobic, the hydrophilic characteristic of hyaluronic acid makes it an interesting carrier to be the shell of the interior drug, forming a micelle.¹²⁹ Micelles have a small size and a higher capacity to load drugs compared with many composites. This core-shell system increases the stability of the drug in blood circulation, and because of the pH-dependent nature, the main part of the drug release would be started after entering into the tumor site (acidic pH environment) in a sustained pattern.¹³² The hydroxyl group of quercetin was linked to adipic dihydrazide-modified hyaluronic acid by Xin Pang *et al.* The developed tumor-targeted bioconjugates exhibited pH-dependent drug release behavior. HA-quercetin micelles inhibited tumor growth in H22 tumor-bearing mice and showed cytotoxicity toward MCF-7 cells four times greater than the free quercetin.¹¹³

5.2.9.2 HA as Drug Carriers

Cytostatic drugs are poorly soluble in water and have high toxicity, making them inconvenient for cancer therapy. Hence, it is important to either conjugate them by covalent links or associate them with entrapment or encapsulation techniques to appropriate carriers. As a result of loading cytostatic drugs, they would have some advantages such as stabilization and

localization in specific body sites.¹³³ Drug delivery systems can improve the solubility/dispersion of the drugs by increasing their stability in blood and reducing the side effects. Hyaluronic acid particles, due to their versatility, have attracted the remarkable attention of researchers in biomedical applications to be used as carriers in drug delivery systems, especially in cancer treatment.¹⁰⁰

In order to get a synergetic anti-tumor effect, in many works, more than one drug is used simultaneously as a co-delivery system. It is possible to use different HA–drug conjugations or even to associate different drugs to the same HA carrier. By combining chemotherapy drugs, not only the therapeutic efficiency would increase in comparison with using them individually, but their side effects would not overlap.¹¹⁴ Furthermore, the cancer chemoresistance, as the main cause of cancer treatment failures, would be surpassed by delivering multiple drugs.¹³⁴

The main goal of using different cytostatic drugs in an HA-based delivery system is to avoid drug incompatibility and provide appropriate pharmacokinetics profiles.^{119,135} For instance, Kelei Hu *et al.*¹¹⁴ synthesized hyaluronic acid–cystamine–polylactic-co-glycolic acid as a nano system to deliver doxorubicin and cyclopamine to bulk breast cancer cells and CD44-overexpressing breast cancer stem cells. As a result of this combination therapy, a synergistic anti-tumor effect was observed, with a lower survival rate of tumors than the mono therapy method. The presence of hyaluronic acid made the therapeutic process targeted toward cancerous cells.

Small interfering RNA (siRNA) molecules have been greatly used in cancer treatment strategies.¹³⁶ At the effector phase, after being initiated, siRNA would be placed in the multi protein RNA-Induced Silencing Complex (RISC); then, an argonate enzyme, as a part of the RISC, breaks mRNA down, stopping the gene expression.¹³⁷ Utilizing siRNA delivery in cancer treatment brings about specified gene suppression in advanced growth stages, with a less expensive process than gene therapy.¹³⁸ For example, Hyukjin Lee *et al.*¹²⁰ fabricated HA nanogels encapsulating green fluorescence protein (GFP) siRNA. It was found that HA nanogels were taken up selectively by HCT-116 cells, which have CD44 receptors on the surface. The gene silencing effect was reduced in the presence of free HA in

the transfection medium as HA nanogels were selectively taken up by HCT-116 cells *via* CD44 receptor-mediated endocytosis.

A novel method of cancer therapy is the co-delivery of siRNA with drugs. Like the co-delivery of multiple drugs, siRNA and the cytostatic drug could be encapsulated either in one vehicle or separately in different carriers and then delivered together. HA has shown great potential to be applied as the carrier of co-delivery of chemotherapeutics and polynucleotide systems. To improve the anti-tumor effect of doxorubicin, Xiongwei Deng *et al.*¹²¹ co-encapsulated miR-34a, as an endogenous tumor suppressive molecule in breast cancer, with the drug into HA–chitosan nanoparticles. miR-34a and doxorubicin were delivered into tumors. Consequently, the expression of non-pump resistance and anti-apoptosis proto-oncogene Bcl-2 was reduced and miR-34a inhibited breast cancer cell migration *via* targeting Notch-1 signaling.

Based on the immune system's role in eliminating exogenous material, vaccinating against cancer is a new strategy developed to treat tumors.¹³⁹ In the presence of exogenous markers, major histocompatibility complex-restricted epitopes are presented by antigen-presenting cells to T cells, helping recognize foreign antigens.¹⁴⁰ Helper and cytotoxic T cell epitopes have already been identified for tumor antigens.¹⁴¹ Tumor-associated antigens, being regarded as recombination proteins, have a low immunogenic level. Augmentation of endogenous immunity stops the proliferation of malignant cells, which is the primary purpose of cancer immunotherapies.¹⁴² Conjugation of HA with antigens would inhibit tumor growth by increasing cytotoxic T cell production, showing more therapeutic efficiency than the administration of the free antigen. In addition, studies showed that different molecular weights of HA do not lead to the same results; for molecular weights up to 200 kDa, the promoted macrophage phenotype would have anti-tumoral properties, but HA with molecular weights more than 800 kDa stimulates the polarization of macrophages.¹⁰¹ Manli Song *et al.*¹⁴³ utilized the ability of HA in re-programming anti-inflammatory, pro-tumoral M2 TAMs to pro-inflammatory, antitumor M1 macrophages to improve the role of manganese dioxide nanoparticles (MnO₂ NPs) for decreasing tumor hypoxia and modulating chemoresistance. The HA coated, mannan conjugated MnO₂ particle (Man–

HA-MnO₂) treatment significantly increased tumor oxygenation while down-regulating hypoxia-inducible factor-1 alpha (HIF-1 α) and vascular endothelial growth factor (VEGF). Moreover, combined delivery of Man-HA-MnO₂ NPs and doxorubicin significantly inhibited tumor growth.

As mentioned before, loading anti-cancer drugs to HA lead to a controlled release pattern, which is a desirable feature in the treatment of tumors. Releasing a drug could happen due to different factors. The first one is some enzymes that are able to be involved in the degradation of HA-coated nanoparticles, which have been associated with specific linkers.⁹⁵ For instance a high level of cathepsin B in tumor endosomes leads to HA being detached from the surface of Au in core-shell Au-HA systems, achieving a burst release of the drug. As a result, the killing of cancer cells would be immensely more significant than that of normal cells. Oyuntuya Gotov *et al.*¹¹⁷ facilitated the release of docetaxel from HA-cleavable peptide-Au nanoparticles by cleaving the peptide, due to the presence of the enzyme cathepsin B in HeLa tumor-bearing mice. The prepared drug-loaded nanoparticles enhanced the cytotoxicity and anti-tumor efficacy compared to the free docetaxel with laser treatment.

Hyaluronidase, a family of enzymes demonstrated in many tumors, is a crucial factor controlling drug release from HA-based composites.^{144,145} In metastasis, the higher expression level of these enzymes compared to the primary tumors leads to the malignancy of tumors.^{146,147} Intracellular hyaluronidase enzymes help the process of HA degradation, which leads to deshielding of the drug-loaded carrier. Mingzhen Zhang *et al.* fabricated a hyaluronidase-responsive targeted drug delivery system, functionalizing mesoporous silica nanoparticles with biotin-modified HA. As *in vitro* analyses demonstrated, the apoptosis efficiency of doxorubicin-loaded biotin-modified HA was more than the free drug.¹¹⁸

5.2.10 HA in Protein and Peptide Delivery

Pavlou and Reichert¹⁴⁸ predicted that the market for protein and peptide delivery would rapidly grow due to the patent expirations happening soon. Bio-actives are essential and non-essential compounds with efficacy on human health, which exist in nature. Peptides and proteins are bioactive

materials. Bio-responsive polymers, such as HA, are polymers with sensitivity to physical, chemical, and biological triggers such as pH, redox, or enzyme.¹⁴⁹ One crucial difference between hyaluronic acid and proteins, such as biopolymers, is the sterically greater rigidity of polysaccharide chains than polypeptide chains. Therefore, hyaluronic acid does not have proteins' ability to form an extra-compact globular structure.⁹

The enhancement of circulation time and stability are the advantages of polymer–protein/peptide conjugates while reducing their toxicity and immunogenicity. Sustained delivery of proteins in parenteral systems can be an advantage of polymer–protein conjugates. A sustained release formulation of growth hormone (SR hGH) was made with the aim of high molecular weight HA (over 2 million Dalton).¹⁵⁰ Hahn *et al.*¹⁵¹ developed a sustained release of spray-dried microparticles of hyaluronic acid and hGH (SR–hGH) under the following conditions: inlet temperature: 100 °C, outlet temperature: 50 °C (keeping the product temperature well below 50 °C for the preservation of intactness of hGH), feeding rate of 1 L h⁻¹. A further vacuum drying procedure was well done for two days after a spray drying cycle. The mean particle size of the SR–hGH product was 5.6 ± 1.0 µm. *In vivo* studies in both beagle dogs and cynomolgus monkeys showed no adverse effects. At the same time, PLGA containing hGH formulations caused severe pain to consumers, due to the large particle size of PLGA and acidic denaturation of hGH. These commercialized HA microparticles were released in 2007 in Korea under the brand name of Declage[®]. Thus, HA-based drug delivery systems worked as a great protein drug reservoir.¹⁵⁰

First-generation protein/peptide–polymer conjugates were pegylated ones. PEG (polyethylene glycol), a non-degradable polymer, showed its efficacy in some protein and peptide conjugates, include Neulasta[®], Oncaspar[®], and Somavert[®]. However, pathological metabolic changes (including vacuolization and lysosomal storage diseases) are associated with repeated administration of non-biodegradable polymers.^{152–155} Consequently, attempts were made to apply biodegradable polymers as components of polymer therapeutics. As is well known, HA is indeed a biocompatible and biodegradable polymer.

Chemical modification of HA and the effect of this modification on HA's body distribution were reviewed by Oh and Park to summarize the

application of this biopolymer in target-specific intracellular transition of nucleotides and long-acting delivery of peptide and protein therapeutics. Elongation of HA is essential as its application in long term delivery can be possible. The short half-life of hyaluronic acid is its main problem in drug delivery.²⁸ The carboxyl and hydroxyl groups of HA are its functional groups for modification strategies including attachment of pendant groups (ester¹⁵⁶ and carbodiimide¹⁵⁷) and cross-linking (with divinyl sulfone,¹⁵⁸ glycidyl ether,¹⁵⁹ or dialdehyde¹⁶⁰) to achieve HA hydrogels. The carboxyl group of HA is one of the targeting sites for its modification because of this recognized fact that carboxyl groups can be identified by HA receptors and hyaluronidase.¹⁶¹ Thus, modification in carboxyl group results in alteration of HA's biological behavior.⁴ Zhong *et al.*¹⁶² concluded that an increasing degree of HA modification led to a delay in the enzymatic degradation of HA derivatives.

For chemical modification of high molecular weight HA, its degradation to a low molecular weight (from several thousand to *ca.* 500 kDa) is the first important step. This molecular weight treatment is possible by heat, pressure, UV, and enzyme.^{163–165} Modification of low molecular weight HA is then chemically achieved in aqueous and organic solvents such as dimethyl sulfoxide (DMSO) and hexafluoroisopropanol.¹⁶⁶ There are various kinds of hyaluronic acid derivatives such as HA–dopamine, HA–tyramine, HA–I, HA–PDPH, HA–SH, HA–aldehyde, HA–NHS, HA–cystamine, HA–HMDA, and HA–ADH.⁴

Encapsulation of erythropoietin (EPO) was also formulated in the cross-linked HA hydrogels.¹⁶⁷ EPO is a 34–39 kDa sialoglycoprotein. The main consumers of this drug are anemic patients who have CKD (chronic kidney disease). Short half-lives of injected EPO were reported by Kampf *et al.*¹⁶⁸ in 1990 following subcutaneous (8.4 h), intravenous (11 h), and intraperitoneal (15 h) administration. Thus, research has been done to improve patient compliance and reduce the dosing frequency of EPO. Among other protein and peptide delivery formulations, hydrogel formulations provide many advantages for the encapsulation of protein and peptide drugs. Hyaluronic, as a hydrophilic, noninflammatory, biocompatible, and biodegradable polymer, acts as an excellent precursor in the formulation of polymer network hydrogels. HA modification is the first

step for the production of cross-linked HA hydrogels. However, the presence of highly alkaline or acidic solutions at elevated temperatures prevents the addition of sensitive components during the production of HA hydrogels. Motokawa *et al.*¹⁶⁷ prepared HA-ADH (adipic acid dihydrazide grafted HA) (M_w of HA = 25 000) according to the method of Luo *et al.*¹⁶⁰ as the first step to achieve the HA-ADH hydrogels. Then, EPO was loaded into the HA-ADH hydrogels by taking advantage of the pK_a difference between hydrazides ($pK_a = 3$) of HA-ADH and the amine group of EPO ($pK_a = 9$), which leads to their selective cross-linking at a pH of 4.8. *In vitro* and *in vivo* (in rats) release studies of EPO showed the hydrogel system's possibility for sustained EPO release.¹⁶⁷ Thus, the three-dimensional structure of the HA hydrogel was considered as a suitable carrier for the sustained release of proteins. Due to the particle size of protein drugs in the range of 3–15 nm, the pore size of HA hydrogels should be approximately 5–25 nm for sustained release of protein drugs by single-file diffusion. Three important factors in HA-hydrogel protein or peptide delivery are (1) controlling the pore size of HA hydrogels in the range of 5–25 nm by changing the cross-linking density of HA, (2) complete degradation of HA hydrogels after protein release and (3) preventing the degradation of proteins during HA hydrogel formation.⁴

Also, Hahn *et al.*¹⁶⁹ studied the sustained injectable hyaluronic acid micro-hydrogels of EPO. This time HA-SH hydrogels were prepared in two steps. HA-ADH was first prepared and then modified into thiolated HA (HA-SH) with Traut's reagent (imminothiolane). The usage of sodium tetrathionate ($M_w = 270.22$), as an accelerating agent, reduced the gelation time from a day to 30 min through the formation of reactive sulfenylthiosulfate intermediates. Additionally, Ellman's assay showed that sodium tetrathionate could also reduce the residual free thiol contents in HA-SH micro-hydrogels.

Furthermore, the formation of droplets during the spray-drying process resulted in a higher concentration of HA-SH on the surface of drops leading to better encapsulation of EPO in the HA micro-hydrogel. The measured mean particle size of HA-SH micro-hydrogels was 2.3 μm , and the water content after spray drying was 14%. Release studies also

confirmed the slow release of EPO encapsulated in HA micro-hydrogels.^{4,169}

Duncan *et al.*¹⁷⁰ described the term “polymer-masking-unmasking protein therapy (PUMPT).” It is a novel approach for polymer–protein modification that masks the protein activity in the bloodstream while enabling restoration of its activity by controlled degradation of the polymer in the target site. For instance, the first attempt to confirm the potential of PUMPT for further application to clinically significant protein therapeutics was trypsin–dextrin conjugation. This conjugate showed the ability of dextrin to mask trypsin activity depending on the molecular weight and degree of succinylation of dextrin. Reinstatement of trypsin activity to a maximum of 92–115% was also possible through incubation with R-amyase. Dextrin was the first biologically inert model polymer to test the PUMPT hypothesis in conjugation with various proteins and peptides, including trypsin, melanocyte-stimulating hormone (MSH),¹⁷⁰ phospholipase A2 (PLA2),¹⁷¹ and epidermal growth factor (EGF).¹⁷²

Furthermore, the potential of HA as a PUMPT component was studied by Gilbert and Duncan.¹⁷³ The suitability of HA for PUMPT application was also explored by Ferguson *et al.*¹⁵² in HA–trypsin and HA–EGF (epidermal growth factor) models. Fortunately, the molecular weight of HA in these conjugates was 90 000 Da, which is vital for stimulation of angiogenesis and inflammation (two essential steps in the wound repair process). Consequently, it can be concluded that conjugating HA to a complementary protein or peptide results in the improvement of their biological effects to promote the healing of chronic wounds. In HA–protein/peptide conjugates, hyaluronidase is the activating enzyme. This enzyme is widespread throughout the body, yet intracellular HA is the most abundant type. Exogenous hyaluronidase is relevant to several clinical conditions (like chronic infections, wounds, and burns). Thus, the usage of such HA–protein conjugates in the wound environment leads to its activation due to the presence of hyaluronidase. Surprisingly, Ferguson *et al.*¹⁵² concluded that the enzymatic activity of the HA–trypsin conjugate had no difference with the free trypsin.

Furthermore, the increased trypsin activity after the unmasking effect of hyaluronidase was another unpredictable result. Thus, it was concluded that

HA conjugation had no impact on the alteration of trypsin's movement, and its activity was not consistently masked.^{152,174} Despite the HA–trypsin conjugate, the HA– α -interferon conjugate demonstrated masking of the protein activity (71% activity of α -interferon remained in the conjugated form). These data show that the PUMPT approach's effectiveness is variable between polymer–protein/peptide conjugates.¹⁵²

Controlled release of proteins from HA and its derivatives was also reviewed by Bayer.³ The unique intrinsic therapeutic properties of HA aimed at the suitability of HA–protein conjugates for sustained release of proteins. However, encapsulating proteins in suitable matrices without loss of stability is a great challenge. Xu *et al.*¹⁷⁵ synthesized heparin (HP)-decorated, hyaluronic acid (HA)-based hydrogel particles (HGPs) using divinyl sulfone as the crosslinker. The method of preparation of HP-decorated, HA-based HGPs was an inverse emulsion polymerization technique. HP was covalently attached to HA and the reason of this attachment was the specific ability of HP to bind bone morphogenetic protein-2 (BMP-2). The higher BMP-2 loading capacity of the hybrid HA/HP hydrogel particles was observed in contrast to pure HA particles.

HA modification for protein conjugation is achieved mostly by the partial oxidation of some sugar units to produce aldehyde groups for the selective conjugation to N-terminal units of the proteins. This conjugation method can be performed in aqueous solutions and at natural pH, which is its great advantage.³⁰

The HA–hGH (human growth hormone) conjugate was prepared by the coupling reaction of HA–ALD (HA–aldehyde) with the N-terminal of the hGH protein. Transdermal application of the HA–hGH conjugate showed that the conjugated form had 10–16 fold higher bioavailability than topically administered free hGH.³⁰

The HA–INF α conjugate was also prepared by HA–ALD conjugation to the N-terminal site of the protein. Intrinsic liver targeting was determined after the administration of dye-labeled HA–INF α in mice. Pharmacokinetic analysis also revealed the prolonged residence time of HA–INF α conjugates, which was dependent on the degree of HA modification. Furthermore, *in vivo* antiviral activity of this conjugate was affirmed by the elevated level of OAS 1 (2',5'-oligoadenylate synthetase 1) in the liver.

Thus, conjugation of INF α resulted in its specific liver targeting and the increasing of its half-life. As was mentioned, selective liver targeting can increase the efficacy of IFN α therapy in hepatitis treatment. However, the level of HA conjugation leads to targeted specific delivery of HA conjugates to the liver. For instance, in the case of HA–INF α conjugates, it was concluded that slightly modified HA targets the liver more specifically than a high loading of HA–IFN α conjugates. Thus, researchers have decided to develop a slightly oxidized HA derivate. To do so, one approach was the periodate oxidation of HA, which resulted in the loss of HA cellular recognition properties due to the opening of sugar rings. Another approach was grafting the aldehyde moieties on HA without distraction of its structural integrity. This approach was implemented by coupling amino glycerol to some HA carboxylic groups. By doing so, only the glycerol unit of HA can be oxidized and the polymer backbone integrity remains unchanged. To make this happen, one equivalent of periodate with respect to the disaccharide unit was used for 5 min. In this short reaction time, only the flexible vicinal diols of glycerol, which have fast oxidation kinetics, will be oxidized without altering the polymer integrity.³⁰

Furthermore, HA–acetal formation was another approach for the introduction of an aldehyde group in the HA backbone without altering its structural integrity. This method is executed with a 4-amino butyraldehyde diethyl acetal spacer, which has a protected aldehyde functionality. The protected functionality means that it can be easily altered to an aldehyde group in acidic conditions for protein conjugation. HA–IFN α was also synthesized using HA–acetal.³⁰

HA–INS (insulin) was also synthesized by the means of HA–acetal and it was examined on the diabetic rats. It was concluded that the HA with a minor degree of INS loading had a longer (up to 6 h) glucose lowering effect. Whereas the effect of free insulin disappeared after one hour. Surprisingly, the HA conjugate with the higher INS loading showed no glucose lowering effect due to its steric entanglement affecting the receptor/protein recognition.³⁰ Alpha-chymotrypsin is a proteolytic pancreatic enzyme, which is inactivated in acidic pH and has the optimum activity in pH 7–9. Protection of this enzyme in acidic pH was achieved by its entrapment in an ethylenediamine derivative of HA, which was

conjugated with a pH sensitive polymer (poly(methacrylic acid) sodium salt). The conjugation was performed by using HA–ethylenediamine as a macro-initiator for the radical polymerization of sodium methacrylate monomers. This polymer precipitates at a pH below 2.5. The precipitation of polymer in acidic pH results in protection of the entrapped protein's structure. As a result, this HA-conjugated pH sensitive polymer can be used in intestine targeted drug delivery.¹⁷⁶

Three functional groups of HA, which are targets for its chemical modification, are the carboxylic, the hydroxyl, and the acetamide groups. Derivatization and crosslinking are two approaches to alter the physicochemical properties of HA. Crosslinking or conjugation of HA can be performed in aqueous or organic solvents, however in aqueous phase some acidic or alkaline reactions lead to HA chain hydrolysis. Thus, the coupling reaction of HA in organic solvents such as dimethylformamide (DMF) or DMSO is also favorable. For solubilization of HA in organic solvents, the conversion of commonly used sodium salt of HA to its acidic form or a tetrabutylammonium (TBA) salt is necessary.³⁰

HA bio-conjugation takes place mostly in aqueous solution in the way of two main derivatization approaches: (1) direct activation of carboxyl group. (2) Oxidation with periodate to form aldehyde groups. The disadvantage of the first approach is the batch-to-batch variation because of the random coupling to protein amino groups and consequently the low homogeneity of the final conjugates.³⁰

In the case of overexpression of vascular endothelial growth factor (VEGF), the use of VEGF receptor antagonists, like Anti-Flt1 peptide, seems to be advantageous. The chemical conjugation of this peptide to HA results in improvement of peptide solubility in aqueous mediums, such as the retina. Furthermore, the mucoadhesive and viscoelastic properties of HA are its main characteristics in successful ocular drug delivery. The chemical conjugation of the HA–Anti-Flt1 peptide was achieved in anhydrous DMSO using benzotriazol-1-yloxy tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as a coupling reagent. The biological activity of anti-Flt1 peptide was preserved by HA and pharmacokinetic analysis revealed the increased residence time of the

HA-peptide conjugate for more than two weeks in laser-induced CNV (retinal choroidal neovascularization) rats.³⁰

Exedin-4 is another anti-diabetic polypeptide that was conjugated to HA in research studies. The current market product of this polypeptide is administered twice daily, owing to its short (60–90 min) half-life. Conjugation of a thiolated exendin-4 to HA-vinyl sulfone derivative by Michael addition showed an increased stability in human blood circulation with respect to free exendin-4. Due to the derivatization of HA to HA-vinyl sulfone at the level of HA carboxylic groups, the recognition of HA by HA receptors and hyaluronidase will be reduced. As a result, the circulation half-life of HA-exendin-4 conjugates will be increased. As an illustration, *in vivo* studies in db/db mice demonstrated the maintenance of the glucose-lowering effect of HA-exendin-4 conjugates for three days, whereas a free exendin-4 effect was retained only for one day.³⁰

CWRYMV peptide was conjugated with aminoethyl methacrylated HA (HA-AEMA) *via* Michael addition. The addition happens between the methacryloyl group of AEMA and the thiol group of cysteine in the peptide. This peptide is an agonistic peptide of the FPRL1 receptor (formyl peptide receptor-like-1), which is helpful in the treatment of inflammatory diseases. The *in vitro* serum stability of the conjugates revealed that the conjugated form was stable for five days without degradation, while the free peptide form decreased by 30% in three days. Furthermore, the activity of the conjugated form was lower than the free peptide form, due to the steric hindrance of the polymer. In addition, after treatment of the conjugated peptide with hyaluronidase, its activity increased.³⁰

HA-peptide conjugates have shown several advantages among the free peptide forms such as: (1) preservation of the conjugated peptide from enzymatic degradations. (2) Preservation of peptide activity by means of hyaluronidase, which degrades HA and allows the recovery of peptide activity. (3) Enhancement of the peptide's serum stability.³⁰

5.2.11 HA in Targeted Drug Delivery Systems

HA is one of the most studied polymers used in targeted drug delivery systems. As a natural component of many human tissues, HA possesses remarkable biocompatibility and biodegradability making it a promising

candidate in the modification of drug delivery systems. Moreover, HA is physiochemically stable, reasonably priced, and available in a wide range of molecular weights. The presence of NH_2 and COOH functional groups in its structure allows the preparation of various derivatives of the polymer which can be used for different purposes. CD44, the main receptor of HA, is highly expressed in many human tissues as well as several types of tumor cells. HA interaction with CD44 receptors is involved in many biological and pathological processes such as inflammation, cancer initiation and progression, wound healing, and morphogenesis. Thus, HA has attracted considerable attention in targeted drug delivery applications. As a targeting ligand, HA is used for main aims such as: (1) to improve tumor cell targeting, (2) to improve cancer stem cell targeting, (3) to improve macrophage targeting, and (4) to reverse multidrug resistance.

5.2.11.1 Receptors of HA

The most widely studied receptor of HA is CD44, which is also the main target of many HA-modified drug delivery systems. CD44 possesses two isoforms, CD44s which is the standard and ubiquitous isoform, and CD44v, the variant isoform, which is specifically overexpressed in cancer cells. A specific isoform of CD44v is the primary receptors overexpressed in many types of cancers, while normal cells do not typically express it. Moreover, CD44 presented on the surface of cancer stem cells helps them in terms of motility abilities. CD44 can also be found on the surface of macrophages. In some kinds of cancers, tumor-associated macrophages play a significant role in cancer progression through the induction of inflammatory responses. Therefore, HA-modified drug delivery systems have a wide variety of applications in the treatment of cancer cells.

In addition to CD44, several other receptors of HA can be found in human tissues. CD168, also known as the receptor of hyaluronan-mediated motility, is overexpressed in certain tumors and is connected with poor prognosis and survival rates. Another known receptor of HA is the HA receptor for endocytosis (HARE) which is responsible for the turnover of systemic HA. Blockage of the HARE receptor inhibits HA clearance in the liver.

5.2.11.2 HA as the Tumor Targeting Moiety

5.2.11.2.1 Tumor Cell Targeting

Perhaps the most widely known application of HA in cancer treatment is its role in targeting the CD44 overexpressing tumor cells. Toward this aim, two main strategies can be used: (1) direct conjugation of HA to the therapeutic agent, and (2) modification of the drug-loaded delivery system with HA. In the first approach, HA is either directly integrated into the anti-tumor agent through covalent bonding or indirectly *via* a cross-linker. The most notable example is ONCOFID-P-B™ which is currently under phase II clinical trial for the treatment of ovarian and bladder cancers. In a recent study, doxorubicin (DOX) and gemcitabine (GEM) were conjugated to the backbone of HA and evaluated *in vivo* with a 4T1 tumor model. Compared to the combination of free drugs, significantly higher tumor growth inhibition was observed with the DOX–GEM–HA polymer. Following 4 injections every 3 days, the tumor size in the group treated with the DOX–GEM–HA polymer was 68 mm³ compared to 500 mm³ in the control group.¹⁷⁷ Selective delivery of paclitaxel (PTX) to tumor cells was achieved using covalent conjugation with HA. A superior inhibition effect was observed in HCT-116 and MCF-7 than normal cells with PTX–HA conjugation.¹⁷⁸

By modifying the backbone of HA, micelles with suitable drug-loading capacities for hydrophobic anti-cancer agents can be prepared. Li *et al.* reported redox-sensitive PTX-micelles based on the HA–deoxycholic acid conjugates that were rapidly disassembled within the tumor cells through the thiol–disulfide exchange reactions with intracellular glutathione. Since glutathione (GSH) is present in higher concentrations in the tumor cytosol than the normal cells, the anti-tumor effects are more specific. These effects are even more pronounced due to the presence of overexpressed CD44 receptors on the studied cell line (MDA-MB-231 cells).¹⁷⁹ PEG-conjugated HA micelles containing irinotecan were able to induce significantly a higher anti-tumor response in different mice colon cancer models.¹⁸⁰

Polymersomes based on the HA can be prepared and loaded with various therapeutic agents to selectively target cancer cells. Paliwal *et al.* synthesized pH-sensitive polymersomes composed of HA to delivery DOX

into MCF-7 cells. pH-sensitive delivery systems are smart systems that can deliver therapeutic agents in a controlled and expected way under certain conditions and in response to specific stimulus such as elevated temperature, ultrasonic waves, pH, ion, and glucose. In their study, the release of DOX was remarkably higher in an acidic environment (pH $\sim$ 5) compared with physiological fluids (pH $\sim$ 7.4). As the tumor environment possesses an acidic nature, pH-sensitive polymerosomes are able to release the drug more specifically within the tumor cells. HA presented in the polymerosomes structure enhanced the anti-tumor effects in CD44 receptor-expressing MCF-7 cells.¹⁸¹

HA hydrogels have been widely studied for different purposes. Owing to the recent development in biomaterials science, HA hydrogels can be prepared with the desired viscoelasticity and 3-D structure to be used in cancer treatment. In one study, HA was functionalized with methacrylate groups to be further conjugated with thiol groups. The results of the study revealed that critical properties of the hydrogel such as adhesion, invasion, and mechanosensing are highly dependent on the presence and the employed concentration of HA within the matrix.¹⁸²

Coating nanoparticles with HA is another attractive strategy in delivering therapeutic agents including genetic molecules and conventional chemotherapy agents to tumor cells. Both organic and inorganic delivery systems can be employed toward this aim. HA-coated liposomes have been extensively studied, especially in cancer treatment.^{183,184} In a study done by Song *et al.*, HA-coated liposomes containing both DOX and PTX were prepared to treat MCF-7 cells with high CD44 receptor expression. Compared to the use of either drugs, the dual-loaded liposomes showed superior cytotoxicity toward breast cancer cells. Furthermore, the HA-coated liposomes were superior to non-modified liposomes in terms of cellular uptake.¹⁸⁵ Another study used HA as the coating agent to modify curcumin-loaded zein nanoparticles for the efficient treatment of CD44-overexpressed CT26 tumor cells. HA-coated nanoparticles demonstrated remarkably higher anti-tumor activity compared to the non-modified nanoparticles.¹⁸⁶ In phototherapy against cancer cells, HA can improve the outcome of the treatment. For example, WS₂ nanodots coated with HA showed improved efficacy against 4T1 cells.¹⁸⁷

5.2.11.2.2 Cancer Stem Cell Targeting

As CD44 is highly expressed on the tumor stem cells, the use of HA as the targeting moiety can significantly enhance the cellular uptake as well as the anti-cancer effects. Coating the PTX-loaded solid lipid nanoparticles (SLNs) with HA was linked with a higher cellular uptake and apoptosis in melanoma stem-like cells. Compared to free drug and non-modified SLNs, HA-SLNs suppressed the tumor growth to a higher amount.¹⁸⁸ Marengo *et al.* synthesized HA-modified liposomes, containing diethyldithiocarbamate-copper ($\text{Cu}(\text{DDC})_2$) to actively target pancreatic cancer stem cells (PCSc). The prepared liposomes profoundly increased the anti-proliferative effects in PCs.¹⁸⁹

5.2.11.2.3 Macrophage Targeting

HA's main receptor, CD44, is also presented on the surface of macrophages and can induce repolarization of macrophages from M2 to M1 type. HA nanoparticles containing miR-29 and miR-155 can promote the repolarization process, and thus enhance the anti-tumor activity of macrophages. In a study conducted by Parayath *et al.*, HA-decorated poly ethylenimine (PEI) was prepared to deliver miR-125b into F4/80^+ peritoneal macrophages. Nanoparticles altered the M1 to M2 macrophage ratio to 6 times the normal level, resulting in significantly improved immunotherapy toward non-small cell lung cancer.¹⁹⁰

5.2.11.2.4 Reversing Multi-drug Resistance

Multi-drug resistance (MDR) in tumor cells occurs through several mechanisms, of which, the overexpression of the corresponding transporters, especially P-glycoprotein (Pgp), is considered the main one. Studies have indicated that interaction of HA with the CD44 receptor can bypass the Pgp and overcome MDR. HA-decorated poly(lactide-co-glycolide) (PLGA) and PEI nanoparticles containing DTX and disulfonated tetraphenyl chlorin were evaluated for the treatment of DTX-resistant HeLa cells. DTX-resistant HeLa cells are highly resistant to free administration of DTX with about an 8-fold higher IC_{50} ($0.093 \mu\text{g mL}^{-1}$) value compared to the non-resistant parent cells ($0.011 \mu\text{g mL}^{-1}$). However, the prepared

nanoparticles were able to induce apoptosis in the resistant cells at much lower drug concentrations compared to the administration of free DTX.¹⁹¹

5.2.12 HA in Self-assembling Systems

Self-assembly is the process of association of individual units into highly arranged structures *via* non-covalent interactions.¹⁹² Self-assembled polymeric nanoparticles composed of biocompatible and amphiphilic polymer conjugates have been investigated for their potential use in drug delivery systems as they can encapsulate a quantity of poorly water-soluble drugs and improve bioavailability, bioactivity, and controlled delivery. By application of lipophilic moieties on the hydrophilic backbone of polymers, the resulting conjugates can readily self-assemble into micelles in aqueous solutions. Their hydrophilic shells provide long circulation in the blood and further increase the probability of reaching the target cells and tissues. HA could be a valuable candidate in the self-assembling systems as HA can interact mostly with CD44 receptors and target the tissues of interest which is a more important issue in cancer treatments.¹⁹³ Moreover, natural polysaccharides such as HA are highly stable, safe, non-toxic, biodegradable, hydrophilic, and biocompatible.¹⁹²

Amphiphilic HA conjugates were synthesized by chemical conjugation of hydrophobic 5 β -cholanolic acid to hydrophilic HA. The conjugated forms were able to form self-assembled stable nanoparticles in an aqueous environment. As a result, the nanoparticles could be efficiently taken up by SCC7 cancer cells which overexpress CD44 receptors and accumulate in tumor tissues. This issue is related to both their prolonged circulation in blood and high affinity toward tumor cells.¹⁹⁴

In a study, self-assembled hyaluronic acid nanoparticles (HA-NPs) were prepared by hydrophobically-modified HA derivatives which could self-assemble into nano-sized particles consisting of hydrophobic multiple cores surrounded by a hydrophilic HA shell. As a result the HA-NPs could selectively target tumor tissues and act as unique drug carriers in cancer therapy.¹⁹³

Hyaluronic acid-benzoyl cysteine derivatives (HA-BC) were synthesized to self-assemble in physiological buffer and produce a chemical hydrogel after oxidation with a fibrillar structure. The HA-BC hydrogel

was examined as a cell scaffold and the results suggested its possible application in tissue engineering.¹⁹⁵

Docetaxel-loaded hyaluronic acid (HA) nano-capsules were prepared by using a self-emulsification process without the need of organic solvents, heat or high shear forces. The nano-capsules exhibited a high docetaxel encapsulation efficiency, adequate stability in plasma, and effective intracellular delivery of docetaxel. Dodecyl side chain-containing HA as the precursor polymer was used in this study to prepare nano-capsules by a spontaneous emulsification process.¹⁹⁶

In another study, hyaluronic acid–glycyrrhetic acid succinate (HSG) nanoparticles were synthesized that could self-assemble into nanoparticles in aqueous solution. By application of HA as the hydrophilic part and glycyrrhetic acid (GA) as the hydrophobic part, the nanoparticles can be prepared by a self-assembly process and also target the liver due to the active targeting capacity originated from both HA and GA. HSG nanoparticles exhibited no significant cytotoxicity and may have great potential as liver-targeted drug carriers.¹⁹⁷

Hyaluronic acid–deoxycholic acid (HD) chemical conjugates were developed as self-assembled nanoparticles delivering doxorubicin as the model drug. HD nanoparticles were more cytotoxic against cancer cells than free doxorubicin at longer incubation periods. As a result the drug loaded HD nanoparticles could improve the solubility and anti-cancer activity of doxorubicin. Moreover, the degree of substitution with deoxycholic acid can affect the particle size, drug encapsulation efficiency, drug release rate, and cell uptake efficiency.¹⁹⁸

Hyaluronic acid–ceramide (HACE)-based nanoparticles were prepared by a self-assembly process to form micellar nanoparticles for the targeted delivery of DOX. A significant inhibitory effect was observed on tumor growth after the injection of DOX-loaded NPs into the B16F10 tumor-bearing mouse model.¹⁹⁹

Another strategy for developing self-assembling characteristics to HA can be achieved by conjugating the positively-charged moieties to facilitate electrostatic interaction with the negatively-charged groups of HA. In this way, nucleic acid-based therapeutics can be easily bound to electropositive moieties on the HA backbone. In a study, HA–poly(ethylene imine)

(HAPEI) blended with HA–poly(ethylene glycol) (HA–PEG) and thiolated HA (HA–SH) in a 1 : 1 : 1 ratio self-assembled to form nanoparticles that encapsulated siRNA molecules with high efficiency. The nanoparticles exhibited high tumor uptake and gene-specific knock down *in vivo* in A549 drug sensitive and A549DDP cisplatin-resistant non-small cell lung adenocarcinoma models.²⁰⁰

5.3 Application of Hyaluronic Acid in Gene Delivery Systems

Gene delivery applies to the introduction of a foreign genetic molecule, such as DNA or RNA, into host cells. The concept of gene delivery dates back to the 1970s, and since then gene delivery has been increasingly utilized in many research and clinical studies as it is potentially able to treat any kind of disorder that is caused by genetic abnormalities. In gene delivery approaches, DNA that is necessary for the production of a specific protein or RNA that is required for normal cell function can be introduced *via* several types of vectors into the intended cells. Thus, in gene delivery, control of the production of vital and cell-building materials is artificially in the hands of humans, and any disorder can be theoretically treated. This is especially important in the case of cancer because in many malignancies, the production of a particular substance is either greatly increased or stopped. Nevertheless, a number of obstacles limit the practical application of the field in the treatment of diseases including cancer. The first issue is the stability of genetic molecules in biological environments. Many DNA and RNA molecules are not able to stabilize *in vivo* for more than a few minutes without the necessary chemical modifications. This instability is due to both physicochemical changes caused by the body's environment and the activity of various types of enzymes that destroy DNAs and RNAs. In general, DNA and RNA destruction in the body occurs in both extracellular and intracellular parts. In the first part, extracellular enzymes and the immune system are the two main factors that significantly reduce the effectiveness of gene delivery, while the main reason for the lack of an appropriate response in the second part is intracellular enzymes as well as the harsh physicochemical conditions of the endosomes/lysosomes. The

next issue is the off-target effects of the genetic molecules on other cells and tissues in the body. Therefore, appropriate gene delivery is possible only when an appropriate strategy can reduce the side effects in other parts of the body and bring genetic therapeutic agents only to the desired area. The last, but not least, issue is the very high cost of many genetic therapies, which practically makes therapeutic interventions limited to laboratory methods and small studies. Therefore, although the benefits and strategies expressed in the field of gene delivery are very tempting and full of potential, until the problems are resolved, it is practically impossible to use them in the treatment of diseases. One of the available solutions is the use of the gene delivery systems, which has led to increased efficiency and reduced costs in the gene delivery field by using different techniques and methods.

The last 20 years have been a remarkable era for the gene delivery field, as different types of gene delivery systems were introduced, improved, and used *in vitro* and *in vivo*. In a large division, gene delivery systems can be divided into two groups of viral and non-viral systems. The first consists of the viruses modified to use the innate ability of infecting cells without causing harm to the host cells. The designed viruses carry the intended genetic molecules, successfully escape the immune system, and specifically enter the cells of interest. Many viral-delivery systems are actually excellent in terms of cell entry and gene transfection efficiency; however, viruses always carry a high risk of pathogenicity and immunogenicity, which limits their use for safety reasons. Non-viral gene delivery systems on the other hand employ natural or synthetic materials to transport genetic molecules. These systems can generally be divided into two categories of organic systems such as lipid-based nanoparticles (NPs), polymer-based NPs, micelles, dendrimers, polysaccharides, albumin and inorganic systems including carbon nanotubes, metal-based NPs, magnetic NPs, and graphenes. Regardless of the type of the delivery system, a suitable delivery approach must meet the following criteria: (1) being able to load, protect, and carry a sufficient amount of the desired genetic molecule, (2) possessing the favorable physicochemical properties and the ability to sufficiently cross the cell membrane, (3) being able to protect the entrapped genetic molecule against external factors such as various physicochemical conditions, enzymes, and the immune system, (4) having the capacity to

escape the endosomes and release the cargo at the site of action, (5) have no or acceptable toxicity profile, and (6) being reasonably priced and affordable. Unfortunately, not all of these features are found in one carrier and often each carrier has its own advantages and drawbacks. Nevertheless, one of the strategies to enhance the efficiency of gene delivery is to utilize external molecular agents to improve the properties of a carrier. Hyaluronic acid is one of the most studied agents that can be used for this aim. As mentioned previously, HA possesses specific physicochemical properties as well as a targeting ability making it as a good candidate in improving the gene delivery vectors. This section focuses on the rational reason of using HA in a gene delivery system along with the recent works.

Since the genetic molecules are highly prone to the degradation, a delivery system must protect the cargo from external factors. Moreover, RNAs and DNAs are negatively-charged and thus the most studied carriers for these molecules are those that are cationic in nature in order to form a strong electrostatic interaction between the vector and the cargo. Cationic polymers (*e.g.* chitosan, polyethyleneimine (PEI), poly(L-lysine) (PLL), and poly amino ester (PAE)), and dendrimers like polyamidoamine (PAMAM) have been extensively studied as the carrier for the delivery of genetic molecules. Nevertheless these polymers need further modification to mask the surface positive charges and increase the circulation time.

PEI–HA conjugates are one of the widely-studied examples. Both high molecular weight HA (HMW-HA) and low molecular weight HA (LMW-HA) can be used for this aim. In a study done by Jiang *et al.*, siRNA delivery using the PEI conjugated to HMW-HA resulted in enhanced silencing effects in cells that have HA receptors but not in those lacking the HA receptors, indicating the role of HA in increasing the siRNA delivery efficiency.²⁰¹ Yao *et al.* used an imine reaction between PEI and periodate-oxidized HA to prepare a PEI-HA copolymer with different molecular weights. The obtained conjugates showed a superior ability to form complexes with DNA. This might be due to the more polar interaction between DNA nucleotides and HA molecules. Moreover, the HA-modified complexes showed lower cytotoxicity and a higher transfection efficiency in HepG2 cells. The presence of HA also resulted in greater accumulation of the nanoparticles in the tumor site compared with the PEI–DNA NPs.²⁰²

Another study used an –SS– bond cross-link to conjugate the HA with PEI. The conjugation was done through cross-linking the PEI with cystamine bisacrylamide and then adding the HA chains *via* reductive amination. PEI-SS-HA polymers were then used to deliver the VEGF siRNA into the tumor site. The study results indicated that HA conjugation significantly enhanced the therapeutic effect of the siRNA delivery.²⁰³

Chitosan–HA conjugates can be prepared through either an ionic gelation method or electrostatic interaction. In the first method, HA alone or with another cross-linker (*e.g.* sodium tripolyphosphate) is added dropwise into a solution of chitosan and left under stirring until nanoparticles are formed. In the second method, HA is added to the formed nanoparticles through the electrostatic interaction. Regardless of the conjugation approach, the addition of the HA to the chitosan chains results in better DNA/RNA complex formation, lower cytotoxicity, and higher cell transfection. In a study conducted by Sato *et al.*, HA with different molecular weights (400, 600, and 1300 kDa) were mixed with a solution of chitosan and pDNA until NPs were formed. Evaluation of the cellular transfection using COS7, B16, and Huh7 cells revealed that HA conjugation led to higher cellular uptake and the transfection efficiency depended on the molecular mass of HA as higher HA molecular weights correlated directly with elevated cellular uptake efficiencies.²⁰⁴ Almalik *et al.* reported higher cellular transfection for HA-coated chitosan-tripolyphosphate (chi-TPP) NPs in CD44-expressing cells. They used HA to coat the prepared chi-TPP NPs by adding the HA to the chi-TPP NP solution indicating that the benefits of HA conjugation do not depend on the conjugation method in chitosan polymers.²⁰⁵

HA-decorated PAMAM/DNA dendrimers (G4 and G5) have shown significantly less cytotoxicity and higher transfection than unmodified PAMAM/DNA dendrimers.²⁰⁶ Urbiola1 *et al.* reported enhanced gene transfection of the pCMV-Luc reporter gene by a PAMAM/DNA dendriplex coated with hyaluronic acid in overexpressing CD44-receptor cancer cells. The prepared polyplexes were also able to efficiently deliver pCMVLuc in the tumors of C57BL/6 animals. The increase in cellular transfection was not observed when a free solution of HA was added to the cells prior to the cell treatment with the NPs.²⁰⁷

The results observed in cationic polymers were also observed in lipid-based systems. Taetz *et al.* prepared a HA-modified DOTAP/DOPE to carry anti-hTERT siRNA into A549 cells. Flow cytometry assay showed that HA-modified liposomes were able to accumulate higher in the CD44-expressing A549 while no clear differences were found when liposomes were added to CD44-deficient Calu-3 cells.²⁰⁸ Run *et al.* prepared polyethylene glycol–HA–liposomes for the delivery of anti-GGCT into drug-resistant MCF-7 breast cancer cells. They utilized amide formation between PEG-NH₂ and the activated carboxylic groups of the HA to covalently conjugate PEG with HA. The resulting PEG–HA was then used to modify DOTAP liposomes to enhance the physicochemical and targeting properties of the NPs. *In vitro* cellular uptake of the NPs showed that PEG–HA–NP had a comparable cellular uptake level compared with Lipofectamine RNAiMAX, a commercially available product designed specifically for the delivery of siRNA and miRNA into all cell types. Antitumor studies on MCF-7/ADR xenograft models on nude mice demonstrated a higher efficiency for PEG–HA–liposomes compared with free siRNA, naked NPs, and HA–NPs.²⁰⁹

The backbone of the HA can be modified with several functional groups, such as acrylates, thiols, or amines to provide crosslinking sites to form hydrogels. Localized gene delivery using hydrogels is one of the novel approaches in tissue regeneration. Tokatlian *et al.* designed 100 and 60 mm porous HA hydrogels loaded with pro-angiogenic (pVEGF) or reporter (pGFPluc) plasmid NPs to induce vascularization in a mouse subcutaneous implant model. After 6 weeks of treatment, all porous hydrogels successfully induced vascularization compared to the control hydrogels.²¹⁰

5.4 Application of Hyaluronic Acid in Imaging

In order to diagnose the sickness or choose the next step of treatment, clinical imaging methods have been developed for the visual representation of the required sites of the body. Nanomaterials, specifically hydrogels, provide useful non-invasive platforms to detect and cure different diseases, such as fluorescence imaging, X-ray micro computed tomography (CT), and magnetic resonance imaging. Since biopolymers have short half-lives, physical or chemical modifications are crucial to increase their stability and

broaden their functionalization for imaging application.¹⁵⁶ Biodegradable and biocompatible polymers such as hyaluronic acid can be highly used in imaging due to their abundant sources and cost-effectiveness with a wide range of molecular weights.²¹¹ The engineered nanostructure of HA nanoparticles makes them attractive candidates for use as carriers for molecular imaging nano probes. In this section, some biomedical applications of HA in imaging will be discussed.

5.4.1 Fluorescence Imaging

A non-invasive method for visualizing molecular processes or structures in living organisms uses fluorescent nanoparticles with the ability to coexist with living cells. Introducing molecules existing in intracellular and extracellular fluids such as HA to fluorescent nanoparticles can increase their water solubility.^{212,213} Near-infrared (650–900 nm) fluorophores are sensitive particles that penetrate into tissues without significant damage.²¹⁴ Combining fluorophores to the hydroxyl and carboxyl groups of hyaluronic acid, which is a part of synovial fluid in joints and tissues, increases nanoparticle biocompatibility.^{215–217} For example, Bin Li *et al.*²¹⁴ prepared HA-coated boron-dipyrromethene derivative nanoparticles to detect tumor cells. They proved the nanoparticle's capability to be used for stable cell imaging, responding to cancer cells with a red and blue fluorescence conversion.

5.4.2 Magnetic Resonance Imaging

For 30 years, Magnetic Resonance Imaging (MRI), due to more tissue-penetration than fluorescence imaging techniques, has been known as a promising candidate to visualize the body's internal structures with more detail.^{218–220} Since this method does not need ionizing radiation usage, it is considered in a non-invasive way. Low sensitivity in MRI is an important issue that highlights utilizing contrast agents (CAs).²²¹ Using an appropriate CA not only leads to a distinctive presentation of healthy and pathological tissues but also improves the intensity of the signal.²²² To determine the contrast enhancement of images because of CA, the 'relaxivity' term is defined as the longitudinal or transverse water relaxation rate constant.

Most CAs have low relaxivity, so they are not accurate enough, and a large amount of them is needed to obtain an appropriate contrast.^{223,224} Also, under ideal operational conditions to gain a high signal to noise ratio and high resolution, more than 3 T magnetic fields are required, in which conventional CAs have no noticeable influence on MRI performance.²²⁵ The other limitation of these CAs is their negligible specificity, bringing about allergic effects.^{222,226} Consequently, it is of importance to design a suitable CA to overcome all the problems mentioned above.

Biopolymer-based carriers of image probes, as non-toxic nanoparticles, present remarkable potential to improve contrast agent relaxivity.^{227,228} Specifically, biodegradable and biocompatible polysaccharides, because of their carboxyl and hydroxyl functional groups, can be easily modified by ionic interactions, covalent crosslinking, ion-complex, or self-assembly.²²⁹ Hyaluronic acid can provide high specificity due to its targeting ability, and improve the safety profile of the CA.^{230,231} Gadolinium diethylene triamine pentaacetic acid (Gd-DTPA) as an MRI contrast agent was mixed with hyaluronic acid by Alfonso Maria Ponsiglione *et al.*²³² to investigate the MRI relaxation enhancement. It was found that hydrogen bonds and coordinate water molecules could be formed because of the interaction between HA and the contrast agent, which affect the relaxometric properties of the diagnosis probe. Another result of their study was the dependence of the interaction between HA and Gd-DTPA on the HA/CA molar ratio.

5.4.3 Theranostic Applications

A new treatment strategy was introduced by Funkhouser, providing concurrent diagnostic and therapeutic entities.²³³ Since this kind of platform uses only one carrier, they are notably cost-effective.²³⁴ In addition, the carriers' ability makes the theranostic systems capable of personalizing the treatment, leading to low side effects.²³⁵ One of the promising candidates of this synergetic technique is HA, which due to the CD44-binding characteristic, has an active targeting ability.^{31,228} HA-based theranostic agents can improve therapeutic outcomes using the electrostatic interaction between HA and superparamagnetic ions, the self-assembling between amphiphilic HA–oleic acid and superparamagnetic ions, the chemical

conjugation of HA onto the surface of tantalum oxide nanoparticles, HA–hydrocaffeic acid with gold nanoparticles, and HA–cholesteryl anchored reduced graphene nanosheets.¹⁰⁰ For instance, Yushen Jin *et al.*²³⁶ conjugated the surface of tantalum oxide nanoparticles with polyethylene glycol, a near-infrared fluorescent dye of Cy7, doxorubicin, and hyaluronic acid to develop a theranostic system. According to in vitro and in vivo experiments, it could enhance fluorescence imaging and CT imaging simultaneously. Furthermore, the platform had the ability of targeted delivery and pH-responsive drug release, exhibiting a high cumulative release rate in acidic microenvironments. For the fabricated nanoparticles, which included HA, the tumor growth inhibition was 4.9% more than the tantalum oxide@Cy7–doxorubicin–polyethylene glycol nanoparticles. This could be related to the targeting ability of HA molecules which facilitated TaOx@Cy7–PEG–HA NPs accumulation in MDA-MB-231 cells.

5.5 Challenges and Opportunities

Hyaluronic acid is one of the most well-known and studied natural polymers in drug and gene delivery systems. Favorable properties of this polymer such as biocompatibility, biodegradability, non-toxicity, reasonable price, high physicochemical stability, and excellent targeting capacities have made HA one of the promising options in the design of gene/drug delivery systems. Various studies have shown the high efficacy of hyaluronic acid with different gene/drug delivery purposes, including cancer treatment. Nevertheless, there are relatively few approved medicines or medical devices containing HA. Also, HA-containing formulations are often not within the top five subjects of the pipeline of the pharmaceutical companies. Part of the problem lies in the intrinsic properties of HA, but the other part relates to studies on the polymer. In many studies, the impact of HA molecular weight was not taken into account and simply ignored. The molecular weight of the HA plays an important role in the toxicity and efficacy of the designed delivery system. While HA with higher molecular weight are relatively safe, HA oligomers can provoke immune responses of the body. In the case of cancer treatment, it is interesting to know that HA with 10–100 disaccharide units might induce tumor growth, whereas HA

containing more than 100 units of disaccharide inhibits tumor growth. Another problem is the lack of accuracy in determining and expressing the amount of hyaluronic acid modification. As shown in the structure of HA, this polymer is abundant in different functional groups of which a specific number of groups are modified depending on the amount of materials used and the reaction conditions. This is true even with the physical interactions including electrostatic interactions. Moreover, the molecular weight and amount of physicochemical modification directly affect the interaction of HA with CD44 receptors and thus impact on the targeting properties of the designed delivery system. When the behavior of a substance is influenced by various factors, and especially when these factors are not well studied by researchers, the observed responses will not be reproducible. As a result, more attention should be paid to the fact that having suitable properties of hyaluronic acid, such as a wide range of molecular weights and different sites for modification, if not carefully identified and reported, will limit the practical use of this polymer in gene/drug delivery systems.

5.6 Conclusion

HA possesses various valuable advantages and characteristics such as biodegradability, biocompatibility, ease of chemical modification, cellular permeability, site-specific interactions with specified receptors, and being involved in inflammation processes and cellular differentiation. These features have made this fabulous polymer an excellent candidate for a vast range of biomedical applications such as drug delivery systems. However, there is the need to keep in mind its limitations such as examining the properties of HA different molecular weights. In future, more intensive experimental and clinical studies should be carried out to explore the diagnostic and therapeutic potential of HA as a combinative agent with different biomolecules or as a nano-carrier itself.

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Chapter 6

Dextran in the Delivery of Therapeutics: Chronicle of the Journey from Preclinical to Clinical Trials

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6.1 Introduction

Drug delivery is the art of conveying a therapeutic entity to its intended site in the body of an organism to produce the desired effect. This is achieved by means of a medium known as drug delivery systems (DDSs), designed to introduce and enhance the efficacy of the said entity by means of regulating its ADME profile as well as functioning as an interface between the drug and the patient.^{1,2} The realm of drug-delivery is ever-evolving in terms of novel research owing to the significant impact it has on the lives of individual's worldwide, accounting for the value of the global pharmaceutical market, currently estimated at \$980 bn annually.³

The primary route of administration of traditional or conventional drug delivery systems (CDDS) (*e.g.*, tablets) is the oral route, upon being delivered through which, they are intended to release the drug tout de suite, promoting its rapid absorption. However,

these characteristics entail certain drawbacks, including necessity of repeated administration which implies that the drug release undergoes peaks and valleys, at times deviating outside the expected range. This might result in either the attainment of a toxic drug concentration or inadequate drug uptake throughout the period of administration of the drug.⁴ In order to surmount these shortcomings, the advancement of an existent drug entity from a traditional delivery system to a more sophisticated, novel drug delivery system (NDDS) may prove to be tremendously beneficial.⁵ NDDSs, such as liposomes, nanoparticles, microspheres, *etc.* serve to enhance the potential of the drug with respect to its efficacy, patient compliance, and safety.⁶ Such systems are designed to mitigate the issues regarding the site and rate of release of the drug, leading to a revival in its performance.⁷

In 1953, with the granting of the Nobel Prize in chemistry to Hermann Staudinger for his efforts to substantiate the presence of “Makromoleküle” lead to the genesis of the field of polymer chemistry, which further lead researchers to view and develop polymers as potential carriers for drug delivery.⁴ For the purposes of developing polymeric drug delivery systems, both natural (hyaluronic acid, arginine, dextran, PLLA, chitosan, *etc.*) and synthetic (PEIs, pHEMA, *etc.*) polymers have been exploited; although there has been a recent shift in focus from synthetic to natural polymers.⁸ The reasons for this change include the similitude of these macromolecules to the constituents of human ECM and their subsequent Increased acceptance by the human body, along with their chemical modifiability, low-toxicity, affordability, and abundant availability.

Natural polymers consist of polysaccharides, lipids, and proteins, among which the former are a subject of scientific interest, thanks to their characteristics of bioactivity and biocompatibility.⁹ Amongst the class of polysaccharides, dextran, a macromolecule synthesized from sucrose by various lactic-acid bacteria, appears to be the palpable choice due to its multiple advantages. Dextran was first introduced by the scientific community about a century ago as a consequence of the blockage of filters caused by the high viscosity of this ‘slime’ and obstruction of the crystallization process in the beet-sugar industries. Scheibler noticed the closeness of the mucilage to starch and dextrin, hence coining the name ‘dextran’. The empirical formula for dextran, now widely known as $(C_6H_{10}O_5)_n$, was also assigned by him. Furthermore, the experiments conducted by Beijerinck (1910) and Hehre (1939 and 1941) laid the foundation of our current understanding of the bacterial sources of dextrans as *Leuconostoc mesenteroides*, *Leuconostoc dextranicum*, and *Streptobacterium dextranicum*, belonging to the family *Lactobacillaceae*.^{10,11}

Dextran (Figure 6.1), a complex branched bio-polysaccharide comprising monomeric units of α -D-glucose is attached by α -(1 $\rightarrow$ 6) glycosidic bonds in the backbone, while branching involves α -(1 $\rightarrow$ 4) or α -(1 $\rightarrow$ 3) glycosidic bonds.¹² Dextrans have a history of being utilized for over 50 years as antithrombotic agents, peripheral flow promoters, and plasma volume expanders; although recent years have witnessed them as subjects to a rejuvenated interest as prospective macromolecular carriers for targeted drug delivery by

passive and active targeting strategies.¹² Its chemical modification can be performed with ease and it also aids in increasing the circulation time of therapeutic agents. Additionally, it exhibits exceptional water solubility, biodegradable, and biocompatible features and serves as a stability-enhancer in drug-delivery systems and furthermore prevents their accumulation in the blood. Particularly, it inhibits the activation of the complement system, hence providing the characteristic of reduced immunogenicity.¹³ These properties make a convincing case for dextran to be an ideal carrier candidate for the development of novel drug delivery systems. All the research hitherto has ushered in a new and exciting era of NDDSs for the formulation of which various natural and synthetic polymers have been experimented with. Among those, dextran is currently on the receiving end of considerable acclaim owing to its assortment of ideal physicochemical properties, all of which serve to entice researchers to consider it as an effective carrier candidate for NDDS.

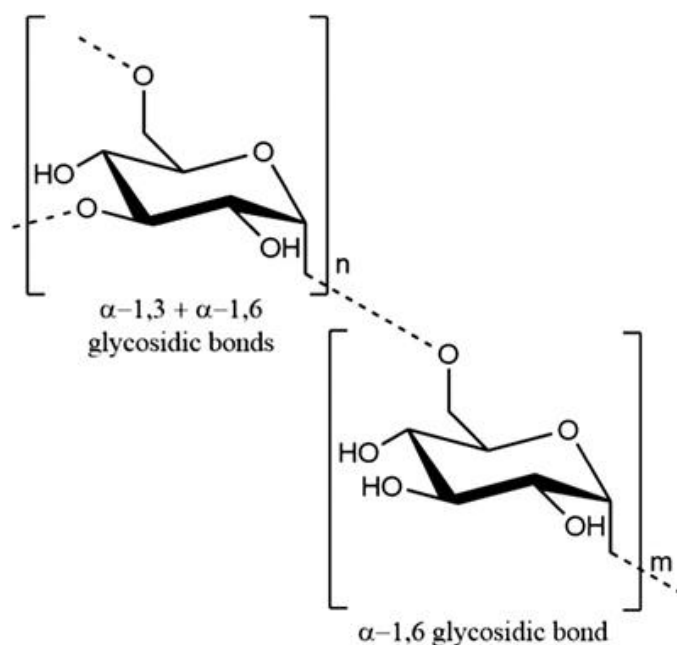


Figure 6.1 Chemical structure of dextran.

The purpose of this chapter is to give a comprehensive overview of dextran: its structural and chemical aspects, application in drug delivery along with a narrative of preclinical and clinical trials conducted on the delivery systems formulated using the macromolecule.

6.2 Dextran: An Extraordinary ‘Slime’ from the Bounty of Nature

6.2.1 Chemistry of Dextran

Dextran, or 2,3,4,5-tetrahydroxy-6-[3,4,5-trihydroxy-6-[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl] oxyhexanal Macrodex (molecular weight, 504.4) can be synthesized from sucrose by transferring d-glucosyl units to the acceptor molecules by the enzyme dextransucrase. Glucose units in dextran are attached via α -1,6 linkages (forming 50–97% of the total number of glycosidic linkages) and α -1,2, α -1,3, and α -1,4 linkages giving rise to branching in the polymer structure. The majority of derivatization processes are carried out using the three hydroxyl groups present in each monomer unit. The most reactive of these groups is the C2 hydroxyl group due to the involvement of C6 groups in forming α -1,6 bonds 14, followed by C4 and C3 hydroxyl groups. The properties of the derivatives of dextrans are affected by the degree of substitution (DS) determined using ^1H NMR, which indicates the number of hydroxyl groups replaced by other moieties in each repeating unit.¹⁴ The water-soluble property is imparted by the presence of linear linkages, usually $\geq 95\%$, rendering this polymer worthy of being exploited for numerous applications, especially with respect to drug delivery.¹⁵ Out of the total linkages forming its branches, 85% have a length of 1–2 glucose units while the other 15% are composed of 33 glucose residues on average.¹⁶

The functional properties of a number of biopolymers may change upon the conversion of the functional groups present in their structures into others; for instance, chitosan loses some of its characteristics when its amine groups are substituted with other groups. However, dextran presents itself as an outlier as tampering with its functional hydroxyl groups does not result in a compromise in its original properties.¹⁷ With a view to improving the characteristics of this molecule and synthesizing its additional derivatives for efficient drug delivery, multiple structural alterations can be effectuated in respect of its temperature sensitivity, ionic strength sensitivity, pH sensitivity, hydrophobicity, and hydrophilicity.¹⁸

6.2.1.1 Dextran Derivatives

Owing to presence of hydroxyl groups, dextran is highly reactive toward chemical manipulations, facilitating the synthesis of a plethora of its derivatives (Figure 6.2) which are further utilized to conjugate various therapeutic entities in order to enhance their ADME properties.¹⁹ Table 6.1 depicts the derivatives of dextran along with their applications in drug delivery.

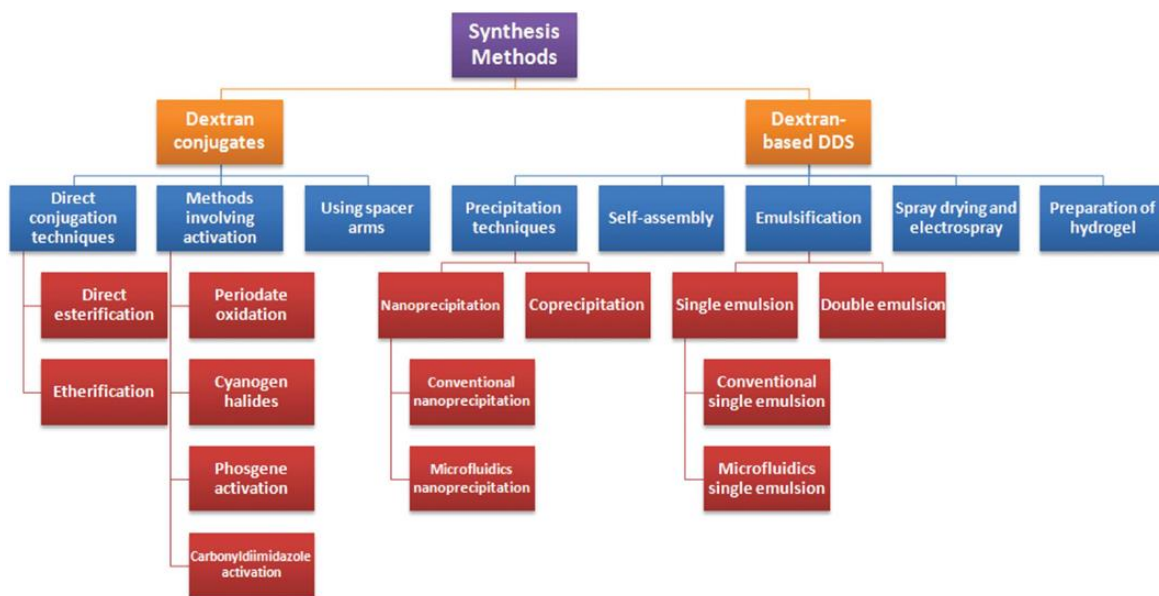


Figure 6.2 Methods for the synthesis of dextran conjugates and dextran-based drug delivery methods.

Table 6.1 Summary of dextran derivatives employed for drug delivery.

Derivative	Therapeutic agents	Ref.
Carboxymethyl dextran	Doxorubicin, paclitaxel, insulin, and lytic peptide	20–23
Dextran sulfate	Oxytocin and vasopressin, curcumin, and silver	24–26
DEAE-D	IVEE vaccine	27
Tosylated dextran	Agmatine	28
Esters of dextran	Lidocaine, ibuprofen and naproxen, catechin, metronidazole	29–32
Ethers of dextran	Cyclosporin A, IgG, BSA, lysozyme	33 and 34
Dialdehyde dextran (oxidation product of dextran)	Hemoglobin, nystatin, isoniazid, and trypsin	35–38
Acetalated dextran	Rapamycin, MOG with DXM, and AR-12	39–41
Carbazate dextran	Doxorubicin	42
Dextran propargyl carbonate	Camptothecin and doxorubicin	43

6.2.1.2 Dextran Conjugates

The process of attaching drugs to carrier molecules, *e.g.* natural or synthetic polysaccharides, lipids, and proteins by means of covalent bonds is termed conjugation.⁴³ Dextran conjugates can be synthesized by two methods: irreversible (*e.g.* dextran–

hormone complexes, dextran–enzyme conjugates, and dextran–metal complexes) and reversible (dextran prodrugs) linking.¹¹ They have been briefly described in the following sections.

6.2.1.2.1 Irreversible Dextran Conjugates

This category involves the irreversible linking of ligand and carrier molecules. These conjugates have shown immense employment in the areas of biotechnology and experimental medicine. They have been briefly described in the following sections.

(a) *Dextran–Metal Complexes*. The conjugation of dextran with heavy metals including antimony and iron is facilely achieved *via* the precipitation of the conjugate in alcohol after the reaction of the polymer with metal salts in aqueous conditions. Antimony–dextran conjugates have been formulated for the treatment of Leishmaniasis in order to maintain sufficient antimony levels in the blood using the carrier properties of dextran after *i.m.* injection.⁴⁴ For circumventing the uneasiness of certain patients regarding the oral delivery of iron, iron–dextran conjugates delivered through a parenteral route have been prepared which further facilitated paid red cell mass regeneration in anemia.⁴⁵

(b) *Dextran–Enzyme Conjugates*. Various enzymes of therapeutic importance, for instance, asparaginase, β -galactosidase, streptokinase, trypsin, α -amylase, catalase, hyaluronidase, α -chymotrypsin, carboxypeptidase, papain, arginase, and NAD⁺ have been conjugated with dextran polymers by taking advantage of multiple mild reactions previously designed to immobilize enzymes on insoluble polysaccharides. The formation of dextran–enzyme conjugates by this technique has augmented the enzymes biological half-life, decreased the likelihood of emanation of allergic reactions after multiple administration cycles, and enhanced their thermal and pH-based stability.⁴⁶

(c) *Dextran Small Molecule Complexes*. Numerous chemical scaffolds have been extensively complexed with charged dextran derivatives such as dextran sulfate, carboxymethyl dextran, and DEAE-D. For example, the conjugate of gentamicin with dextran sulfate⁴⁷ has reportedly resulted in decreased nephrotoxicity of the drug and that of isometamidium has led to ameliorated therapeutic efficacy.¹¹

6.2.1.2.2 Reversible Dextran Conjugates (Prodrugs)

The formation of dextran-based prodrugs can be executed by the utilization of different techniques.¹¹ These include:

1. Direct linkage of the drug molecule to the polymer chain, restricting the drug-release mechanism from the dextran chain to only pH-dependent hydrolysis.
2. Linking of the drug to dextran *via* an intercalated spacer arm, the variability of the terminal functional group and length of which allows for the drug attachment to be carried out through a range of covalent bonds and the circumvention of steric hindrance of the bulky polymer with regard to the cleavage of the bond and subsequent release of the drug respectively.
3. Attachment of a modulator ligand, an inactive chemical molecule to the prodrug which aids in the optimization of the physicochemical characteristics of the conjugate, including partition coefficient, solubility, and electric charge, enhancing the overall disposition of the prodrug.
4. Incorporation of a homing device, or a target-specific ligand such as a hormone or antibody that will ensure the transport of the prodrug selectively to the target tissue of the drug. Examples of the drugs formulated into dextran-based prodrugs include budesonide,⁴⁸ doxorubicin, camptothecin,⁴² *etc.*

6.2.2 Physicochemical Properties of Dextran That Contribute to Its Superior Carrier Status

Dextran has a plethora of physicochemical properties that make them efficient carrier candidates for drug delivery. Depending on the source, the structure and properties including solubility, degree of branching, physiological action, glycosidic links, and optical activity may vary.¹¹ Furthermore, the molecular weight distribution of dextran is influenced by its source and this, coupled with the degree of branching, has a profound effect on the physicochemical properties of the polysaccharide. For instance, as per reports, the water solubility of the macromolecule is inversely proportional to the degree of branching, which in turn ranges from 0.5–60% depending upon the source. Indeed, dextrans with >43% 1,3- α linkage (*i.e.* >43% branching) are adjudged water insoluble; although commercially prepared dextrans are characterized by high water solubility, attributed to a low degree of branching, namely 0.5%.³³ They are soluble in water (> 30 mg ml⁻¹), dimethylsulfoxide, 4-methylmorpholine-4-oxide, formamide, glycerol, hexamethylphosphamide, and ethylene glycol and insoluble in acetone and alcohol.^{11,49} Interestingly, their behavior in formamide as well as in aqueous environments resembles that of a random coil. The aqueous solubility of dextrans is ascribed to their capacity to form hydrogen bonds due to the presence of multiple hydroxyl groups in their structure.⁵⁰

The physiological behavior of dextrans is notably influenced by another physicochemical property labeled as the degree of polydispersity, which is high in native dextrans but can be reduced by their partial depolymerization and fractionation. The commercially available dextrans for research possess a polydispersity of ~ 1.5 and molecular weight of 4–200 kDa. For plasma volume expansion and/or blood flow enhancement purposes, 10% solution containing 40 kDa (low MW) dextran and 6% solution containing 70 kDa dextran (high MW) are available. Dextran 70 kDa is used for the restoration and maintenance of blood volume in situations of extensive burns, shock, and hemorrhage, whereas dextran 40 kDa functions to treat vascular occlusion and enhance blood flow in capillaries. The respective ranges (40 kDa and 70 kDa between 10–90 kDa and 20–200 kDa) of MWs utilized as clinical dextran.¹³ It is worth mentioning that even though the likelihood of occurrence of side effects in parenteral dextran therapy is low, dextran-induced anaphylactic reactions have been observed to occur in rare circumstances. However, this can be avoided by injecting Promiten, a monovalent hapten dextran, as a counter therapy in advance.¹⁶

The viscosity of the solutions of dextran can be demonstrated as a function of the average molecular weight of the polymer, its concentration, and temperature. These solutions reportedly act as Newtonian fluids, as the highest molecular weight is observed to be independent of shear rate at various concentrations. The presence of immense branching in the structure of this polymer leads to compactness in the molecular chains.⁵¹ Hence these chains accumulate in the shape of a rigid sphere in aqueous environments. The absence of consistency in the estimation of intrinsic viscosity of water–dextran systems emphasizes the tremendous impact that temperature and solvent have on these systems. With an increase in the temperature, the polymer structure undergoes compaction, leading to impediment in the flow as a result of increased utilization of energy.⁴⁹ The application of heat facilitates decreased interaction of polymer chains with the solvent, adapting a contracted conformation due to decreased hydrogen bonding and overall increasing the intermolecular linkages with other polymer chains in the solution.⁵¹

The swelling property of dextran, primarily with reference to hydrogels, has a significant impact on the rate of drug release, as it regulates the diffusion rate into the hydrogel matrix as well as the diffusion and dissolution of the drug out of the matrix.⁵² The swelling behavior of dextran chains in the presence of aqueous medium is dependent on the type of network structure, influenced by the crosslinking density, hydrophilicity (controlled by the number of hydroxyl groups in the structure), and the average MW present between two crosslinks.⁵³ The development of the hydrogel network is also a result of the physical interaction occurring between the polymer scaffolds *via* hydrogen bonds and side chains. The hydrophilicity of dextran is responsible for its property of burst-swelling, a characteristic of hydrogels.⁵⁴ The dissociation of polymer chains is partly obstructed by the presence of hydrogen bonds, enabling water to enter the system, as well as hindering the drug effusion resulting in prolonged release of the drug from the hydrogel. The property of prolonged release may serve to enhance the half-life and

bioavailability of the drug, while simultaneously minimizing adverse effects.⁵⁵ This phenomenon has been further extrapolated to synthesize “smart” thermosensitive hydrogel systems capable of controlling drug release as a response to temperature changes occurring in the external environment by virtue of swelling and deswelling.⁵⁶

Due to their high reactivity toward various chemical reactions, dextrans have the potential to be widely employed as drug-carriers by linking them to the polymer structure. However, the functional group they possess in abundance is the hydroxyl group, imposing a limit to the number of drugs that can be potentially attached directly to the dextran matrix. This drawback can be overcome by applying the spacer arm technique which enables the attachment of drugs consisting of varied functional groups to the polysaccharide chain. Providing an edge over the other carriers, the homogenous scaffold of dextran makes the characterization of synthesized conjugates relatively easy. Additionally, dextrans retain sufficient stability even after chemical modifications. Interestingly, dextrans are also autoclavable. High solubility in water can be retained (up to E-20% w/w) even after substantial ligand attachment.¹⁶ These desirable physicochemical characteristics accompanied by high affordability and an extensive clinical usage in the past deem dextrans worthy of being exploited as drug carriers.

6.2.3 Methods of Synthesis

The following sections cover the theoretical bases of the preparation techniques of dextran-based conjugates and their subsequent delivery systems in brief.

6.2.3.1 Dextran Conjugates

Here, the most long-standing and relevant methods for the conjugation of various drugs and proteins with dextran polymers have been succinctly described. A few examples of the application of these techniques have been described below.

6.2.3.1.1 Direct Conjugation Techniques

These are composed of rather straightforward procedures that yield dextran-based drug conjugates containing practically unaltered polymer besides the attachment of the intended drug/protein molecule.¹³

(a) *Direct Esterification.* Esters of dextrans can be synthesized on account of the copious presence of hydroxyl groups in the polymer structure making the conjugation of the drugs containing –COOH groups easily attainable, as evidenced by the production of ester prodrugs of dextrans with multiple NSAIDs by Larsen *et al.*, including ibuprofen, fenoprofen, ketoprofen, indomethacin,⁵⁷ naproxen,⁵⁸ and diclofenac. These conjugates result from the reaction between the hydroxyl and carboxylic acid groups of dextran and NSAID molecules, respectively, in the presence of *N,N'*-

dicyclohexylcarbodiimide.⁵⁹ The second kind of dextran ester that is produced is the copolymer of dextran ester and olefin compound. Self-assembly of dextran esters into nanospheres containing multiple groups such as propyl-, furoyl-, acetyl-, and pyroglutamyl- as substituents at various degrees of substitution has also been performed.⁶⁰

(b) *Etherification*. Irreversible etherification of dextran is carried out via nucleophilic substitution that utilizes aliphatic and aromatic epoxides, halides, or sulfates. On the other hand, reversible dextran ethers are synthesized by the addition of methyl vinyl sulfone, acrylamide, and acrylonitrile; α,β -unsaturated reagents using Michael addition method.⁶⁰

6.2.3.1.2 Conjugation Methods Involving Activation of Dextran

The attachment of drugs that cannot be conjugated to dextran directly, for instance, drugs devoid of a carboxylic acid functional group, necessitates the activation of dextran prior to the reaction between the drug and the carrier. The activation methods for dextrans that are currently in widespread practice are summarized below.

(a) *Periodate Oxidation*. The primary application of this method is to conjugate proteins and enzymes to polysaccharides such as dextran by the conversion of selected hydroxyl groups to aldehyde groups by the use of sodium periodate and reacting the formed dialdehyde dextran with the amine groups present in the protein chain, resulting in the formation of a Schiff base. However, due to the instability of the base and its tendency to release the drug quicker than the desired amount of time, sodium borohydride is used to reduce it and thus create a relatively stable bond leading to prolonged release of the protein.¹³ Proteins including insulin,⁶¹ chymotrypsin,⁶² and soybean trypsin inhibitor⁶³ and daunomycin⁶⁴ have been successfully coupled to dextran using periodate oxidation. This method is preferred for the synthesis of dextran–protein conjugation as it aids in retaining a substantial amount of pharmacological activity of the protein even after conjugation with the carrier molecule.⁶⁵

(b) *Cyanogen Halides*. This method is predominantly employed for the purpose of conjugation of dextrans with drugs (*e.g.* ampicillin) and proteins (*e.g.* aprotinin, insulin, and soybean trypsin inhibitor) containing amino groups.¹³ The reaction of various cyanogen halides (cyanogen chloride, bromide, and iodide) with an insoluble derivative of dextran, namely Sephadex was investigated previously,⁶⁶ and their endeavor led to the revelation that the degree of activation of Sephadex caused by cyanogen

halide influenced the degree of substitution of therapeutic entity on the polymer. They also determined that the degree of convenience was highest in the case of cyanogen bromide when compared to the rest of the halides. Although this technique came into the spotlight upon its advent, its use in contemporary times has dwindled as there is a generally imperfect understanding of this method in addition to which, it is slated to simultaneously produce various drug–dextran conjugates having dissimilar structures.¹⁶

(c) *Phosgene Activation*. Phosgene activation is intended for the conjugation of polysaccharides such as dextran with drugs containing hydroxyl ($-OH$) and amine ($-NH_2$) moieties as functional groups. The hydroxyl groups of the drugs form carbonate esters whereas the amines form carbamate esters upon reaction with activated dextran, created by the activation of hydroxyl groups of the polymer using phosgene. The carbamate ester-based dextran conjugates display a decrease in toxicity as well as an increase in the duration of activity as compared to the free drug. In a function different from the one previously mentioned, chlorocarbonate dextran ester synthesized from a drug can be additionally utilized for the production of enzyme conjugates, as intermediate products. There have been instances of use of estrone or testosterone⁶⁷ to synthesize carbonate esters based on HPC and of insulin⁶⁸ to prepare dextran carbamate esters using phosgene activation. However the application of this method is accompanied by disadvantages such as multiple side reactions and the formation of byproducts.¹⁶

(d) *Carbonyldiimidazole Activation*. Carbonyldiimidazole can be used to bring about the activation of the hydroxyl groups of dextran, to which ethylenediamine is linked in order to introduce amine groups into the chain that are further reacted with materials such as PEG, the hydroxyl groups of which are activated using a succinimidyl compound.⁶⁹ This method of dextran activation also finds application in the coupling of dextran with cromoglycic acid.¹⁶

6.2.3.1.3 Techniques Making Use of Spacer Arms

The usage of linker molecules connecting the therapeutic agent to a dextran structure, *i.e.* a spacer, serves two purposes. Fundamentally, these molecules, especially the ones with a simple and shorter structure, function to link the drug lacking the appropriate functional group to react with the hydroxyl groups of dextran to the carrier molecule. For instance, glutaric or succinic acid has been used as a spacer arm in order to couple dexamethasone

to methylprednisolone.⁷⁰ In other instances though, the spacer arm technique is also exploited for the purpose of pH- or enzyme-based control of the release of the drug from the delivery system. An example of this application is the dependence of the release rate and consequent antitumor action of mitomycin C on the spacer arm length; in that, the shorter the length, the quicker the drug release.⁶⁵ More intelligent spacer arm designs with the ability to target even specific cells or cellular components to release the drug in, nonetheless, are in imminent development.¹³

6.2.3.2 Dextran-based Drug Delivery Systems

There are various techniques for the formulation of drug delivery systems containing micro- and nano-particles of dextran (Figures 6.2 and 6.3). These are briefly discussed in the following sections.

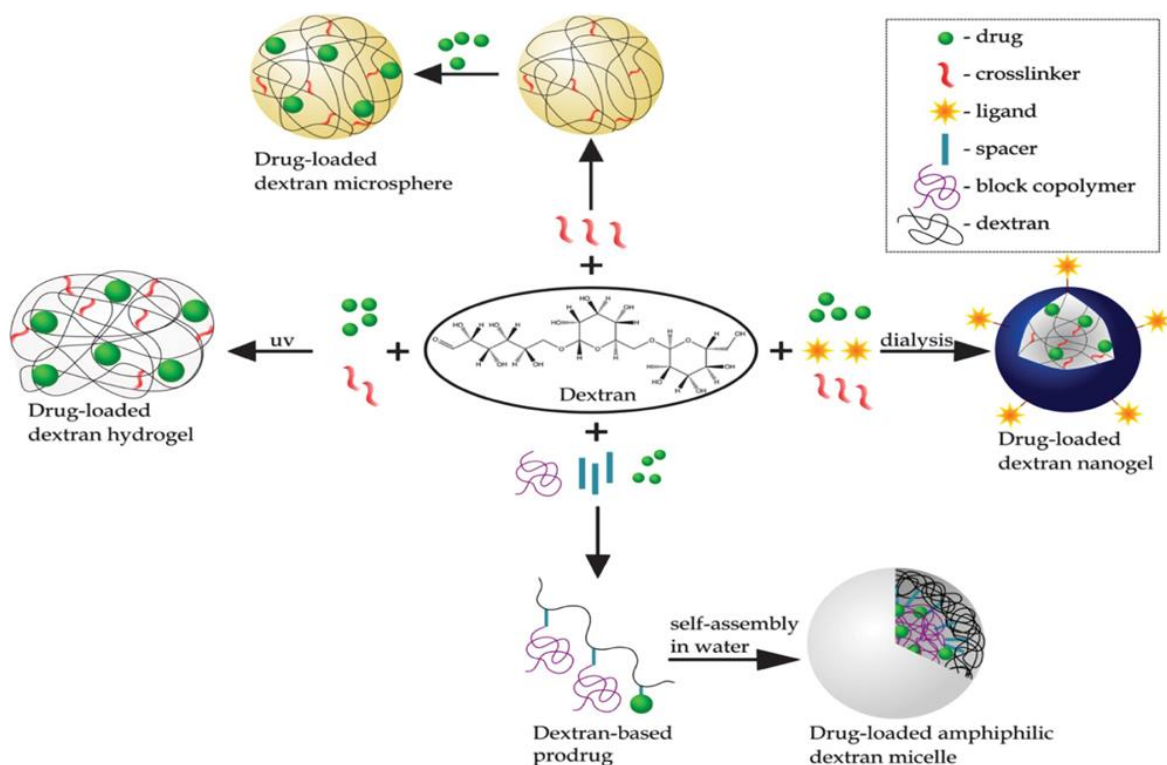


Figure 6.3 Schematic representation of the synthesis of dextran-based drug delivery systems.

6.2.3.2.1 Precipitation Techniques

(a) *Nanoprecipitation*. Nanoprecipitation is the bottom-up approach for the synthesis of nanoparticles when the polymer is subjected to the following conditions: alteration in the salt concentration and pH value, or interaction with an anti-solvent.^{71,72} This technique follows four steps, the first one being the creation of a supersaturated polymer solution followed by the step of nucleation and the subsequent blockage of the same resulting from

rapid reduction of the supersaturated condition finally leading to deposition of the monomers and the development of the precipitates on the surface of the generated nuclei.⁷³ In the following subsections, the most popular techniques of nanoprecipitation namely conventional, microfluidics nanoprecipitation, and coprecipitation of dextran-based nanoparticles have been summarized.

Conventional Nanoprecipitation. Ac-DEX has been most notably used in the preparation of nanoparticles using conventional nanoprecipitation, which simply conveys the addition of the organic solution of Ac-DEX to miscible water-based antisolvent subjected to swift magnetic stirring conditions.⁷⁴ For instance, the dissolution of Ac-DEX in acetone and the regulated addition of this solution to an aqueous solution of polyvinyl alcohol *via* a syringe by Shkodra-Paula *et al.* resulted in the precipitation of Ac-DEX nanoparticles with an average size of 210 nm.⁷⁵ Furthermore, the employment of this technique in the preparation of relatively complex core-shell nanoparticles has also reportedly yielded positive results. For example, Kong *et al.* achieved the synthesis of core-shell Ac-DEX nanoparticles by combining the shell of Ac-DEX with core calcium carbonate particles which can be exploited for loading antibodies, drugs such as doxorubicin, and gold nanorods to attain simultaneous imaging and PTT along with drug delivery.⁷⁶

Microfluidics Nanoprecipitation. Conventional nanoprecipitation, even though a reliable approach, involves a longer process time due to magnetic stirring resulting in inhomogeneities in the surface polymer deposition of nuclei and consequent polymer aggregation.⁷⁷ Hence, batch-to-batch variation and polydispersity can be observed in the synthesized nanoparticles.^{78,79} This has caused increased interest in the formulation of nanoparticles using microfluidics nanoprecipitation as this technique has been shown to expedite the mixing of solvent inside minuscule capillary networks, thus making it capable of synthesizing polymer nanoparticles in a reproducible and controlled manner to the extent of producing 700 g of particles everyday using a single device. Santos' lab has conceived glass-capillary based microfluidic chips with a co-flow design to facilitate nanoprecipitation of Ac-DEX, forming smaller monodisperse particles.⁷⁷

(b) *Coprecipitation.* Co-precipitation, while not being devoid of drawbacks such as aggregation, poor stability of the product and magnetization loss; is an excellent, economic, and easy method primarily used for the formulation of IONPs.⁸⁰ A useful strategy to overcome the challenges associated with IONPs is the formation of an organic compound coat held together by covalent interactions over them that is impenetrable to oxygen, prohibiting its contact with the surface of the nanoparticles and alteration of the magnetic properties. Dextran is the appropriate choice for the formulation of this coat with regards to its biocompatibility as well as safety; the vast array of its applications in terms of coating the IONPs acting as evidence for the same.^{81–83} This technique involves simultaneous precipitation of aqueous solutions of dextran together with ferric and ferrous salts brought about by adding ammonium hydroxide.

6.2.3.2.2 Self-assembly

Nanoparticles, particularly micelles, are composed of an extensively organized structure produced by the interactions of their disordered components through a process termed self-assembly. These interactions are usually directed by non-covalent linkages such as hydrogen bonds, van der Waals, hydrophobic, and electrostatic interactions. Micelles synthesized by self-assembly consist of hydrophilic heads and hydrophobic tails aggregating as a unit, interacting with the surrounding medium; typically aqueous, in which case the heads face outwards and enhance the micelles' stability whereas the tails create a hydrophobic core that encapsulates a lipophilic drug. Current research has been concentrated on the application of amphiphilic copolymers in the formulation of polymeric micelles,^{84,85} with the copolymers of dextran being synthesized by etherification and esterification of the molecule, among which esters are preferred. This is due to the fact that a multitude of compounds are available for the completion of the reaction including acid anhydrides, imidazoles, carboxylic acids, and succinates. For instance, deoxycholic acid was chemically coupled to a dextran ester and self-assembled into micelles for the encapsulation of doxorubicin⁴¹ and similarly, CuAAC click chemistry was utilized to graft PNBA on an alkylated dextran. This photosensitive glycopolymer was then made to self-assemble into micelles.⁸⁶

6.2.3.2.3 Emulsification

Nanoemulsions have particularly been conceived for the enhancement of the delivery of drugs characterized by instability, low efficacy, and aqueous solubility. In the case of nanoemulsions, the emulsification process is optimized to retain the particle size of the droplets in the range of 20–600 nm.⁸⁷ Nanoemulsions can be categorized as w/o (water in oil), o/w (oil in water), as well as bi-continuous nanoemulsions. Over the last ten years, amphiphilic dextran copolymers have been employed as stabilizers in the formulation of various kinds of nanoemulsions,^{88,89} one of which is acetalated-dextran on account of the uncomplicated procedure involved in its synthesis, its biodegradability, sensitivity to pH, and biocompatibility.

(a) *Single Emulsion*. As the name suggests, two liquids of immiscible nature, one of which is dispersed in the other as droplets stabilized by the use of an emulsifier constitute a single emulsion. Most of them are available in the o/w form and can be obtained via methods necessitating high energy consumption such as sonication, typically applied in a laboratory setting.⁹⁰ This process involves the disorganization and eventual mixing of the immiscible layers as a consequence of the energy utilized. However, it is not thermodynamically favored as emulsification causes amplification of the surface area, hence requiring energy to initiate the disruption of droplets.⁷⁹

Conventional Single Emulsion. This process is usually opted for the synthesis of micro- and nano-particles of Ac-DEX, especially in the context of positively-charged derivatives of Ac-DEX. Polymers such as Ac-DEX^{91,92} are solubilized in the oil phase (*e.g.* DCM), to which a surfactant (*e.g.* PVA)-carrying aqueous phase is added and energy is applied to convert the phases into an emulsion.⁹² Attainment of particles having low PDI values and

an average diameter of 250 nm (in cases of Putrescine-conjugated acetalated-dextran and SpAcDEX, positively-charged derivatives) is quite possible provided that the process variables including temperature, time, and intensity of sonication as well as continuous and dispersed phase volumes are optimized.^{91,92} This method is appropriate in order to load drugs with a high degree of hydrophobicity.

Microfluidics Single Emulsion. In spite of the adjustability of the product characteristics in conventional single emulsion at a laboratory or small scale, scaling up of the techniques proves to be an issue. Thus microfluidics single emulsion has been garnering popularity as a remedy to the problems arising in the previous technique.⁷⁴ This method offers a higher degree of control over the emulsification process, facilitating homogeneity and a narrow PDI in the product and involves the dissolution of the surfactant-containing aqueous phase in one channel along with that of the polymer in oil phase in the other.⁷⁹ The formulation of emulsion occurs when the flow of the oil phase is disrupted at a T-junction by the shear stress or cumulated pressure established by the movement of the aqueous phase. Reportedly, Ac-DEX-based microspheres have been formulated by this technique in which the organic phase and surfactant respectively were ethyl acetate and poloxamer 407.⁹³

(b) *Double Emulsion.* This can be described as a principal emulsion droplet enclosed inside a second emulsion globule. The form of double emulsions that finds the most application in the pharmaceutical industry is the w/o/w (water in oil in water) type emulsion ultimately adequate for the encasing of a hydrophilic drug or in the synthesis of core-shell nanoparticles.^{94,95} The conventional process for the formulation of double emulsion includes the solubilization of a hydrophilic therapeutic molecule in an aqueous discontinuous phase which is subjected to sonication in an organic dispersion medium. This medium is later given the same treatment in an aqueous phase forming the second emulsion where the organic liquid is the dispersed phase and the second aqueous liquid is then the continuous phase. Finally, solvent evaporation is utilized to obtain the particles.⁷⁴ Ac-DEX-based nanoparticles encapsulating ovalbumin have been previously produced by this technique with an average size of 270 nm.⁹⁶ SpAcDEX, particularly, is apt for the loading of siRNA and it shows a high encapsulation efficiency upon preparation of the nanoparticles by double emulsion.⁹⁷ However, the encapsulation efficiency of this technique is lower as compared to that using other drugs, as being hydrophilic, it has lost the secondary aqueous phase.

6.2.3.2.4 *Spray Drying and Electrospray*

Spray drying is a technique to produce powders and has found application in the encapsulation of drugs in the pharmaceutical sector. This method uses atomization to break up the volatile solution containing polymer and drug into small sized droplets into a chamber supplied with flow of a hot gas. This sequence ultimately dries the droplets forming the powder by the transfer of the solvent molecules into the gas prior to collection in filters or cyclones.⁹⁸ Physical characteristics of the powder can be optimized

by the regulation of certain process variables including the temperature of the chamber and the gas, the optimization setup and the concentration of the drug present in the solution. Deep lung administration of rifampicin was facilitated by the development of a microscale dry powder inhaler using acetalated dextran by Kadota *et al.* The suspension was produced by the mixing of aqueous acetalated dextran and an ethanolic solution of rifampicin which was then spray-dried using a Buchi Mini Spray Dryer. This approach enhanced the delivery of rifampicin as the inhaled powder was deposited directly on the mucosa of the lung.⁹⁹ Furthermore, this method is extremely versatile and can be used in combination with nanoprecipitation and single immersion techniques. For instance, single emulsion and spray drying have been used to synthesize acetalated dextran nanoparticles loaded with paclitaxel.¹⁰⁰ Spray drying is also suitable for scaling up, synthesizing aerosol powders at a micro-scale characterized by a low aqueous content, facilitating long term storage and enhanced encapsulation efficiency.⁷⁴

Akin to spray drying, electrospraying is a method that functions by atomization of drug polymer solution into droplets in order to obtain drug-encapsulated powder, however, the droplets are dispersed into the air in the direction of an opposite charged solid or liquid collector.¹⁰¹ Characteristics of the final formulation including size and morphology of the particles are governed by process parameters such as flow rate, temperature, charge, viscosity, collector distance, and the solvent used.¹⁰² In the case of dextran-derived drug delivery systems, this technique has been particularly used to synthesize Ac-DEX-based micro- and nano-particles,¹⁰² e.g. the synthesis of Ac-DEX microparticles of resiquimod by Duong *et al.* using a single capillary electrospray instrument, in which ethanolic solution of Ac-DEX was mixed with Tween 80 and the drug and the process resulted in spherical particles. Additionally, electrospraying has reportedly led to a 10-fold enhancement in the encapsulation efficiency of the microparticles as compared to a single emulsion technique owing to minimization of the amount of drug diffused into the aqueous phase resulting in drug loss.¹⁰³

6.2.3.2.5 Preparation of Hydrogel

A hydrogel can be understood as a three-dimensional matrix containing polymer chains of a hydrophilic nature that are linked together by the virtue of a crosslinker. According to Warren *et al.*, the network formed by the crosslinker enables the hydrogel to maintain structural integrity even in aqueous environments and absorb water so much so that the eventual structure is comprised of over 90% water.¹⁰⁴ The crosslinker molecule attaches polymeric chains through covalent bonds or non-covalent interactions like electrostatic and hydrophobic interactions, hydrogen bonds, and van der Waals interactions *etc.* Techniques used for the synthesis of hydrogels include physical, chemical, and radiation crosslinking and grafting polymerization. Recently, Qu *et al.* synthesized an electro-responsive dextran-based hydrogel by mixing the polymer with an electroactive aniline trimer followed by the addition of hexamethylenediisocyanate for its cross-linking

properties. This reaction is responsible for the cross-linked network formation between the polymer chains by using the amine moiety of HDI and hydroxyl group of dextran.¹⁰⁵

6.3 Preclinical Success Stories

Preclinical trials of dextran-based formulations were conducted on experimental animals like rats, mice, and rabbits (Table 6.2). Dextran acts as an effective carrier for drug delivery, however it needs further refinement for it to be considered as a suitable medicament delivery.

Table 6.2 Preclinical trials conducted on dextran-based formulations.

Study design	Dosage form	Dose-dosage regimen	Inference	Ref.
Investigation of the functional neovascularization of the formulation with immobilized GFs in female Lewis rats	Three hydrogels were formulated such as 40% Dex-AE/60% PEGDA (40/60), 60% Dex-AE/40% PEGDA (60/40), and 80% Dex-AE/20% PEGDA (80/20)	Preparation of disk-shaped (2 mm thick 4 mm diameter) Dex-AE/PEGDA hydrogels was done in a sterile environment. For each time point, two sample implantations were done per rat.	The 80/20 hydrogel exhibited higher gel fragmentation and a thicker macrophage layer whereas 60/40 and 40/60 hydrogels showed restricted and negligible penetration respectively. The 80/20 hydrogel was found to be more apt for study hereafter as a vascular growth scaffold since the same pattern was observed for the whole transplantation.	105
Preclinical studies to determine radiation doses and biodistribution in rats and rabbits	[^{99m} Tc] DTPA–mannosyl–dextran	Toxicological study – single dose given in the right-hand footpad under observation for 2 weeks. Group1 (CG) – saline dose Group 2 (LDG) – 0.7 nmol kg^{−1}	Toxicological outcome: both animals are well tolerant at all dosage levels of DTPA–mannosyl–dextran. Biodistribution outcome: clearance from	106

of body wt.
Group 3 (HDG)
– 7.0 nmol kg⁻¹
of body wt.

Biodistribution studies: study duration: 18 h – evaluation of rabbits 15 min (2F) and 1 h or 3 h (2M and 2F) post injection. Animals were administered 0.1 nmole, 0.050 ml of the formulation in the right foot pad.

the injection site
– biological $t_{1/2}$
of 2.21 0.27 h.
Diffusion of the formulation into circulating blood and lymph channels after injection.
Adverse effects: mild hepatocyte hypertrophy observed in rabbits, otherwise formulation well-tolerated.

Comparison of the formulations in Molt-4 tumor induced in 5- to 6-week-old female BALB/c Nu/Nu athymic mice	Examined efficacy of DOX alone, T101 and DOX covalently linked to dextran, T101 + DOX blended together and injected, separate injection of T101 and DOX, and nonspecific murine IgG2A plus DOX blended together	Initial animal trials consist of 6 therapeutic arms; I.P. injection 2 times administration using single injection OR five I.P. injection daily. T101 500 µg, 3.6 µg of DOX 500 µg or T101–DOX–dextran IC containing 3.6 µg of DOX.	T101 + DOX exhibited higher antitumor efficacy than T101–DOX–dextran 1C formulation in similar concentration and much greater effect than only T101, DOX alone, individual injection of T101 + DOX, or DOX + nonspecific IgG2A blended together.
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Examining the cellular uptake and accumulation in tumor cells <i>in vivo</i> in Gliosarcoma-induced rats	LCDIO particles	10 mg kg ⁻¹ iron equivalent rhodamine labeled LCDIO was administered by an IV route and after 24 h of which 0.2% Hoechst 33258 dye (100 µl) was given.	In brain tumors 0.11% ± 0.06 LCDIO accumulation was found which can be significantly detected by MRI. Similarly preferential uptake of LCDIO was observed in macrophages (21.0% ± 3.1), tumor cells (49.0% ± 4.6), and active angiogenesis performing endothelial cells (6.5% ± 1.4), in proportion to the rate of cellular proliferation.	108
Release of hGH from microspheres	Two separate batches of hGH-loaded dex-HEM microspheres were formulated respectively as the initial 50% water concentration and 10% protein load w/w dry weight (ATM1) or 10% of hGH (ATM 2) initial protein loading. A placebo formulation	Group 1 – 0.1 PBS once daily for 5 days continuously for 4 weeks. Group 2 – 166 mg of total hGH 190 was given (0.1 ml PBS consisting of 8.3 mg hGH) for 5 days continuously for 4 weeks. Group 3 – initially 0.1 ml of microsphere suspension in PBS was given which releases 232 mg of hGH. Group 4 – dosage was given initially 0.1 ml of microspheres suspended in PBS which releases 156 mg of hGH at ATM.	In dwarf mice, data of the increase in body-length and in body-weight was compared between a single subcutaneous injection of hGH loaded dex-HEMA microspheres and daily subcutaneous injections of hGH in saline during a period of 4 weeks. Antibodies specific to hGH were observed 4 weeks later from the beginning of the hGH treatment.	109

was prepared
of dex-HEMA
DS16 with an
initial
aqueous
content of
50%.

Pit-1 lacking Snell
dwarf mice

6.4 Clinical Trials of Dextran as a Carrier

Clinical trials of dextran-based formulations were conducted on patients and healthy volunteers (Table 6.3). It is evident that even though the innovation of novel dextran-based drug delivery systems is on the rise, only a small number of them are actually being tested on humans. Moreover, most of the systems that reach the level of clinical trials do not yield optimistic results as their progress in other arenas is often eclipsed by their multiple adverse effects.

Table 6.3 Clinical trials conducted on dextran-based drug delivery systems.

Formulation	Nature of the trial	Dose-dosage regimen	Inference	Ref.
Dextran-40 and pentoxifylline infusion in SHL and AAT	Randomized, placebo controlled, double blinded trial in 382 patients.	The patients in the dextran-pentoxifylline group (group 3; and group 1 and 2 were given placebo and pentoxifylline treatments, respectively) received the 500 ml infusions at varied rates along with additional ampoules and tablets of pentoxifylline	Adverse effects: Asthma (1 ×), hypertension (3 ×), headache (5 ×), vomiting (9 ×), and nausea (12 ×). Frequency of side effects: group 3 > group 1. Absolute hearing gain observed: 19 dB (avg). Relative hearing gain observed: AAT – 40% and SHL – 35%. Comparison between groups: group 3 (dextran-pentoxifylline) was inferior to groups 1 (placebo) and 2 (pentoxifylline) in the mean hearing gain in both SHL and AAT treatments.	110

		e throughout the trial (35 days).	Dextran may have resulted in this observation as groups 2 and 3 had the same dosage of pentoxifylline. The only difference was the presence of dextran in group 3.	
Conjugate of dextran with doxorubicin (AD-70) in solid tumor	Pharmacokinetic and phase I clinical trial in 13 patients	Single dose administered intravenously every 21–28 days. Total 24 courses. Doses: 40 mg m ⁻² DOXeq – 2 patients, 12.5 mg m ⁻² DOXeq – 5 patients, 20 mg m ⁻² DOXeq – 5 patients, and 25 mg m ⁻² DOXeq – 1 patient.	Adverse effects: The side effects of this intervention include thrombocytopenia (at 40, 12.5, and 20 mg m ⁻² DOXeq), hepatotoxicity (at 40 and 40 mg m ⁻² DOXeq), cardiotoxicity (at 40 mg m ⁻² DOXeq), local phlebitis, increase in alkaline phosphatase, vomiting, and malignant fibrous histiocytoma (at 12.5 mg m ⁻² DOXeq). MTD of AD-70: 40 mg m ⁻² DOXeq. Pharmacokinetics: C _{max} for total DOX – 12.3 µg ml ⁻¹ (at 40 mg m ⁻² DOXeq) and AUC – 28.83–80.21 µg ml ⁻¹ h ⁻¹ . Dose-dependent t _{1/2} .	111
Iron dextran and ferric carboxymal dose in iron deficiency anemia	Phase 3b, randomized, open label, multicenter trial to compare the safety and efficacy of the medications in 160 patients	Weekly administration of a single dose (15 mg per kg body weight up to 750 mg) continued until the	Adverse effects: FCM group patients – 0% immune system disorders and 7.3% skin-related disorders. DEX group patients – 10.3% immune system disorders and 24.4% skin-related	112

attainment of the total iron requirement or 2250 mg (the maximum).

disorders. FCM was responsible for less side effects. Those in the DEX group were mainly immune response-based.

Comparison of the treatments: both equally effective for IDA, increasing Hb by 2.5 g dL⁻¹ in about a month (from 9.5 g dL⁻¹ to 12 g dL⁻¹) after the first dose. However, FCM was reported to better restore iron by increasing ferritin levels than iron dextran.

Sustained release hGH-loaded dex-HEMA microspheres	Trial to evaluate <i>in vitro</i> – <i>in vivo</i> correlation in 10 healthy men between 50 and 80 years of age	Subjects were administered four, approx. 1.1 ml (75 mg microspheres per ml) abdominal s.c. injections equivalent to 14 mg hGH on day 2 of the trial. Follow up was taken at various points until the 90 day mark.	The preclinical trial on the same formulation conducted in mice led to a good <i>in vitro</i> – <i>in vivo</i> correlation of effects of the hGH released from the microspheres. Similarly, a good IVIVC was obtained between the hGH serum concentrations calculated from the <i>in vitro</i> data and measured in the volunteers upon the administration of the microspheres. The peak concentrations reached were higher, 1–2.5 ng ml ⁻¹ obtained after gradual increase over 7–8 days after administration. Moreover, increased serum concentrations of IGF-I and IGFBP-3 biomarkers were observed.
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6.5 Future Perspectives

The arsenal of attributes possessed by dextran making it an ideal carrier and the resultant opportunities apropos drug delivery are the essence of this review. However, despite all its

virtues, the polymer is not without its shortcomings which can be surmounted with research and innovation.¹¹³

It is known that the multiple sources of dextran govern its structural properties such as molecular weight and degree of branching that subsequently affect its biological activity.¹¹⁴ The *in vivo* fate of dextran as a function of these properties remains a relatively uncharted domain to date. A clear understanding of the changes in the properties of the polysaccharide including its metabolism, structure, as well as the role of the target site of the conjugated drug is imperative for the design of more effective novel dextran-based drug delivery systems and to upgrade the ones already in existence.¹⁷

Coming from microbial sources, possible contamination with immunogenic endotoxins diminishing the efficacy of the polymer is a concern, calling for the need of a regulated process to achieve its appropriate separation and purification.¹¹⁵ The tremendous scope of the potential role played by dextrans in the recent future has been indicated by contemporary studies that focus on their utilization in the field of drug delivery, although a large portion of these experiments have been performed on experimental animals and have witnessed no promotion of the developed conjugates. A reason posited for the lack of enthusiasm toward them is the requirement of advanced bioanalytical techniques arising from the regulatory obligations regarding specific characterization of these conjugates along with their components as well as their *in vivo* disposition.¹³ Even though dextran is considered superior as compared to other polymers in terms of safety owing to its natural origin, the unexpected development of instances of hepatotoxicity and thrombocytopenia in the participants of a Phase I clinical trial of dextran and doxorubicin conjugate, probably due to the reticuloendothelial system-based uptake of the polymer has cast a doubt over its non-toxic nature.¹¹²

This necessitates more toxicity studies pertaining to dextran conjugates. Apprehension about the use of dextran in clinical settings has further increased due to the cases of anaphylactic reactions to high molecular weight dextrans, even though their occurrence is low.¹³

Recent years have beheld an amplified interest in the application of dextran conjugates, however, their numerous properties have not been completely comprehended thus far. The knowledge gaps in the field may be filled by the determination of the mode of action and the molecular target of the drug. This may be achieved by exploitation of the carrier properties of dextran including the shielding of the drug from premature biodegradation in the body by regulating the degradation property of the dextran chain.¹¹ The commercial use of dextran-based drug delivery systems has been further hampered by the arduous and costly nature of the chemical reactions responsible for their synthesis, resulting in problems with the scale-up and commercialization of these formulations.⁶⁰ Despite the aforementioned limitations, dextran-based delivery systems have the potential to be clinically viable. The resolution of these issues would be a huge step toward introducing them on the commercial platform.

6.6 Conclusion

Drug delivery is a fast-paced, ever-evolving domain of research as it directly affects the entire human race. Our understanding of delivery systems has progressed significantly from the time we first started ingesting herbal remedies and applying cataplasms¹¹⁶ in order to treat malaise and wounds. The current technology has achieved the remarkable feat of operating at micro- and nano-scales with the purpose of advancing the efficacy of the drugs administered to individuals. The arrival of state-of-the-art research into the area of drug delivery has led to the delineation of novel approaches for disease management. One of the strategies gaining particular interest is the formation of conjugates of the drug with carriers such as natural as well as synthetic proteins and polysaccharides by covalent coupling.

Dextran, a member of natural polysaccharides, has a critical role in targeted delivery and controlled release of the therapeutic entity. It has been extensively studied to enhance the efficacy of the drug by greatly increasing its concentration in the targeted tissue along with mitigating the damage caused by the drug to the essential organs and minimizing the overall side effects on the body. The versatility of the polymer provides the advantage of biocompatibility as an addition to the ability to manipulate its dimensions and degradability as per requirement. Moreover, the cost-effectiveness of dextran serves as a benefit from an economic perspective. The precision of the regulatability of the sequence of dextrans structure by biosynthetic and chemical techniques has been improved due to the increased conception and development of the polysaccharide and its derivatives. The usage of dextran in drug delivery may increase significantly with structural modifications to enhance its pH, temperature, and ionic strength sensitivities along with its hydrophilicity and lipophilicity.

Furthermore, the formulation of dextran into well-grounded systems such as liposomes, polymeric micelles, and nanoparticles has yielded positive results, as per expectations. These formulations are worthwhile as their flexible nature makes them suitable for both oral and parenteral administration. In this context, the ability of dextran to solubilize hydrophobic drugs is of vital importance as it results in a long circulating carrier system capable of being retained into the target tissue for an extended period. Initial studies conducted on dextrans mainly focused on the delivery of small molecules, however, the potential of the polymer to deliver protein and peptide drugs have been highlighted quite recently. It has also been reported to be an effective non-viral vector for the cellular delivery of plasmid DNA.⁶⁰ Considering the untapped potential of dextran along with all its prominent contributions as a carrier molecule, it is imperative for the scientific community to explore it further and pave the way for more breakthroughs in the field of drug delivery.

Abbreviations

ADME Absorption, distribution, metabolism, and excretion

NDDS	Novel drug delivery systems
PLLA	Poly(l-lactic acid)
PEI	Poly(ethylenimine)
pHEMA	Poly(2-hydroxyethyl methacrylate)
ECM	Extracellular matrix
IVEE	Formalin-inactivated Venezuelan equine encephalomyelitis virus vaccine
BSA	Bovine serum albumin
IgG	Immunoglobulin G
MOG	Myelin oligodendrocyte glycoprotein
DXM	Dexamethasone
NAD	Nicotinamide adenine dinucleotide
DEAE-D	Diethylaminoethyl dextran
NSAID	Non-steroidal anti-inflammatory drug
HPC	Hydroxypropyl cellulose
PEG	Polyethylene glycol
Ac-DEX	Acetalated dextran
PTT	Photothermal therapy
IONP	Iron-oxide nanoparticles
CuAAC	Copper(i)-catalyzed alkyne–azide cycloaddition
PNBA	Poly(<i>o</i> -nitrobenzyl acrylate)
DCM	Dichloromethane
PVA	Polyvinyl alcohol
PDI	Polydispersity index
SpAcDEX	Spirocyclic-acetalated dextran
Dex-AE	Dextran-allylisocyanate-ethylamine
PEGDA	PEG diacrylate
GF	Growth factor
DTPA	Diethylenetriaminepentaacetic acid
CG	Control group
LDG	Low dose group
HDG	High dose group
DOX	Doxorubicin
LCDIO	Long-circulating dextran-coated iron oxide
dex-HEMA	Hydroxyethylmethacrylated dextran
hGH	Human growth hormone
ATM	Animal trial material
PBS	Phosphate-buffered saline
SHL	Sudden hearing loss
AAT	Acute acoustic trauma
MTD	Maximum tolerated dose
AUC	Area under curve
FCM	Ferric carboxymaltose

IDA	Iron deficiency anemia
IGF-1	Insulin-like growth factor 1
IGFBP-3	Insulin-like growth factor binding protein-3

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Chapter 7

Guar Gum-based Biomaterials in the Delivery of Therapeutics

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7.1 Introduction

7.1.1 Gums/Polysaccharides as a Choice for Therapeutics

Since human civilization began, mankind has been looking to natural resources to meet day-to-day needs. The process of exploration of natural raw materials led to the discovery of the therapeutic properties of various natural plant materials which were used as such or as an extract from various plant parts to cater to various ailments. Later on, as civilization progressed, agriculture flourished and various techniques relying on different plant resources were modified. One such important resource is natural gums, which come out as exudates from plants and are collected and used in various applications, one of which is therapeutic usage. Knowledge about the gums expanded with time and their classification based on yield. Their superiority in application, natural habitat, climatic conditions, *etc.* was also studied to grow them profitably and also drew recognition due to their eco-friendly high cultivation potential, inexpensive, edible standards,

and much more. Some such gums include guar gum, gum ghatti, gums derived from acacia, locust bean, konjac, Tara, Tragacanth, Jhingan, xanthan, and carrageenan, and many more examples are available.

On comparing the structure of these gums, it is seen that these are often present in the form of polymeric units formed by the addition of similar repetitive monomeric monosaccharide units connected by glucosidic bonds.¹ The monosaccharide units (or glycans) are mainly composed of three/four to nine carbon atoms. They differ in size in different ways and stereochemical properties in one or more carbons to impart their unique configuration.

Polysaccharides are classified as homopolysaccharides or heteropolysaccharides depending upon the one or more than one kind of monosaccharide unit. Polysaccharides offer a unique structure in the form of a straight backbone of monosaccharide chain or a branched form. These backbone types of structures offer high surface area and can be modified by various methods to suit our requirements. One major application is the medicinal use as excipients, in the form of drug delivery in various modes.²⁻⁷ Polysaccharides have acquired this status due to the following reasons.

Polysaccharide's accessibility from renewable sources and abundance received the spotlight by researchers for their structure, chemistry, properties, extraction procedures, applications, cost, toxicity status, *etc.* before putting them into any use.^{6,8} Generally, all polysaccharides are biodegradable and biocompatible.⁹ They can be used in their native forms as such or may be modified using a variety of standard chemical and enzymatic procedures.¹⁰ Many natural polysaccharides even in their native forms exhibit many properties that could be used to develop a large number of drug delivery systems.¹¹ For example, ionic polysaccharides exhibit strong pH and ion sensitivity and therefore they can be used to design dynamic drug delivery systems.¹² The incorporation of various functional groups in polysaccharide units not only improves their efficiency compared to their native counterparts but also builds their interaction with other biological macromolecules like proteins or peptides which further escalate their application potential to another level.¹³ Therefore, it becomes imperative that polysaccharides have emerged as a natural choice toward

therapeutic applications like drug delivery that can deliver an enclosed drug at a specified target and time by planning its drug reaction, concerning a particular physical activity.

These polysaccharides or gums are present in many heterogeneous structures in nature. We disintegrate these natural structures to use them as such, or restructure them in any other suitable form. This may be done by endless ways of modification by incorporating desired functional groups or attaching required monomers over them. Therefore, we have the option of using these polysaccharides or gums in their native forms or by modifying them in an endless number of ways. Although the natural precursors also show good results in applications, their efficiency increases manifold upon modification. These modification techniques may be through existing techniques or as combination techniques or possibly an entirely new system of synthesis may be used. The different yields of modified products, their efficacy in the application depend upon the type of modification technique involved, type of functional groups or monomers attached, and other factors like ambient conditions, a reaction setup, commercialization potential, *etc.* So, it is required to intensely research all the possible combinations of reaction, process, conditions to result in the best possible grade of material, and its best efficiency toward any intended application.

7.2 Why Guar Gum?

Guar gum or a cluster bean of botanical name *Cyamopsis tetragonolobus*, which belongs to the Leguminosae family is a common agricultural crop grown annually and found in tropical and subtropical climates in north-western and western India, Pakistan, South Africa, Australia, and some regions of the USA.¹⁷ India and Pakistan themselves produce 90% of the total amount of guar gum used and with 80% of the producibility, India is the largest trading country in the world.^{14,15} Guar gum, also called *Cyamopsis* gum or Guaranis, a natural non-ionic polysaccharide that is soluble in water and found in guar plants, is the cheapest source of galactomannan.¹⁶ It is obtained from the seed endosperm of *Cyamopsis psoraloides*, that has a plant height of 0.6 m and 5–12.5 cm length pod size.^{16,17} The gum obtained from seed endosperm is odorless, appears

whitish-yellow, and has a high molecular weight. It is insoluble in organic solvents such as alcohols and ketones except in formamide. Water is the only significant solvent in which it dissolves to form a viscous solution.^{14,15,18–20} The solubility is due to the presence of an 85% water-soluble part *i.e.* mannose and galactose units containing a hydroxyl group.²¹ Structurally, it consists of a long polymeric chain of α -mannose units linked by $\beta(1 \rightarrow 4)$ glycosidic linkage attached with the side chain of α -galactose units alternatively through $\alpha(1 \rightarrow 6)$ glycosidic linkage^{14,15,18–20,22–24} in a 1 : 2 ratio, as shown in Figure 7.1.

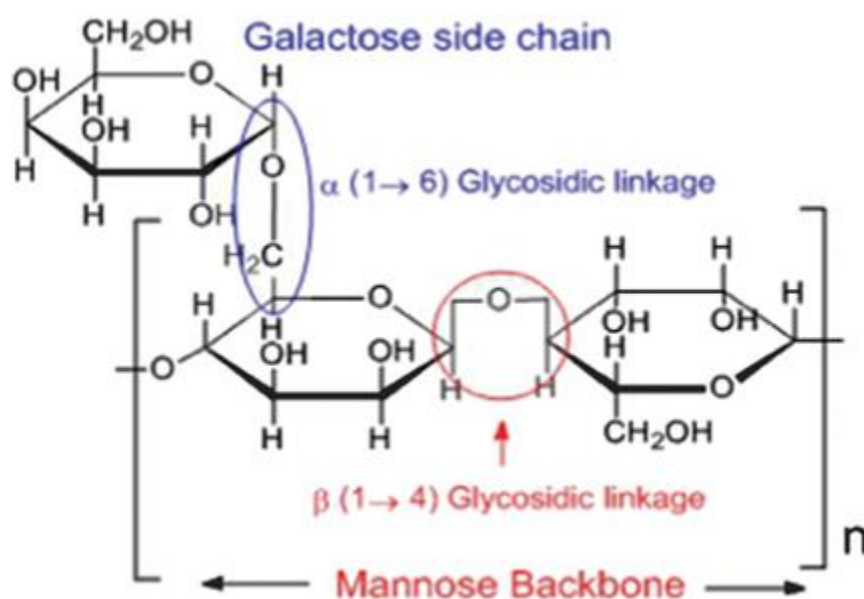


Figure 7.1 Structure of guar gum.

In addition to carbon, hydrogen, and oxygen it contains small amounts of nitrogen and phosphorus. Also, the presence of hydroxyl groups in the polysaccharide backbone opens up the opportunity for its modification to synthesize many derivatives for diverse industrial applications.^{24–26}

Various chemical tests (methylation, acid hydrolysis), analytical techniques (SEM, NMR, IR-spectroscopy), biological tests (hydrolysis of selective enzymes), physical tests (stress–strain measurement) are used to determine the chemistry and structure of polysaccharides.^{27,28} All its chemical properties depend on its constituents and structural features *i.e.*

the presence of a hydroxyl group, degree of polymerization, chain length, *etc.*²⁹ From advanced analytical techniques the average molecular mass of guar gum is determined and found to be between 10^6 to 2×10^6 g mol⁻¹.

Guar gum attains a high viscosity in less time in both hot and cold water even at room temperature, whereas other natural gums require prolonged heating to attain the same characteristic solution properties. The rate of hydration and viscosity of guar gum is dependent on features such as pH, temperature, concentration, dispersion, and the presence of other foreign substances. Owing to its high viscosity, gelling, non-toxic, and easily available properties, it is a popular choice among researchers for use in various industrial applications. Guar gum attains the highest hydration at pH 8–9 and the lowest at pH <4 and >10.¹⁸ Temperature is also another factor that influences the viscosity of guar gum. At high-temperature hydration, the rate is more than compared to low temperature but prolonged heating affects the interaction of guar gum and water molecules by changing the order of water molecules around guar gum and thus results in a decrease in the rate of hydration and viscosity.^{30,31} The presence of OH groups helps guar gum to form hydrogen bonds with a water molecule in an aqueous solution. Other than mannose units, galactose units also expose the hydroxyl group which results to form a highly hydrated colloidal system.¹⁸ The presence of sugar and salt molecules also affects the hydration rate and properties of guar gum. Sugar molecules compete with guar gum for hydration and thus decrease its viscosity whereas salt molecules increase the intermolecular interaction and thereby enhance the rate of hydration. Due to this excellent property guar gum is used as a dispersant and coagulant in organic and inorganic solutions respectively.³²

7.2.1 Guar Gum Processing

India is the leading country and exporter of guar gum with more than 85% of the global production. It is obtained from guar seeds, a legume plant that is cultivated and grown in the semi-arid regions of Gujarat, Rajasthan, and Haryana. Guar seeds are subjected to many mechanical processes such as screening, milling, dehusking, roasting, polishing, and grinding, *etc.* for the extraction of guar gum powder. The stepwise procedure is shown in [Figure 7.2](#).

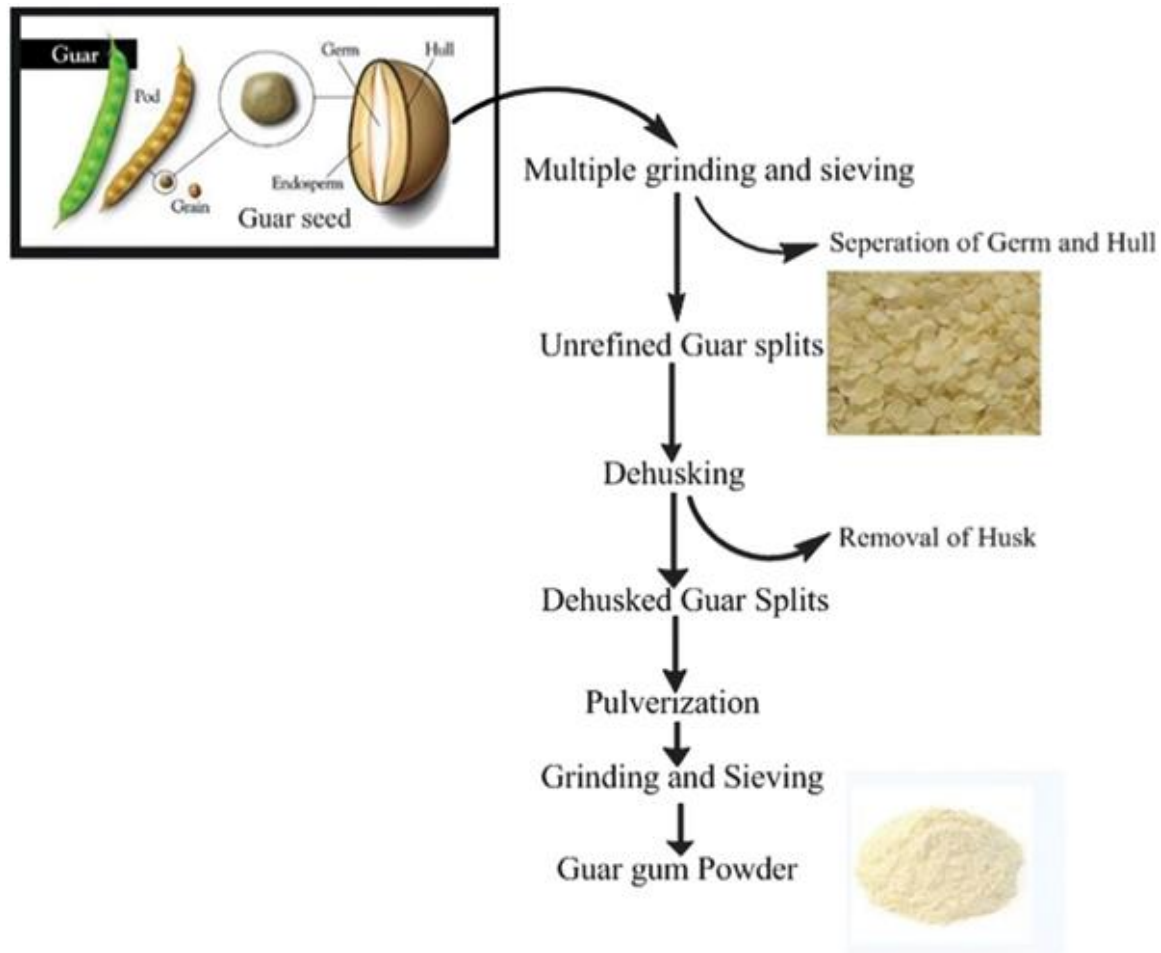


Figure 7.2 Processing of guar gum.

7.2.1.1 *Split Formation*

Guar seeds are collected, processed, and split into two halves. Furthermore, the germ and the hull are scrapped off mechanically to obtain the endosperm.

7.2.1.2 *Screening of Split*

It is one of the important steps in guar gum processing to obtain guar gum powder. In this step, split forms are screened and selected for further processes. The selected splits are cleaned and allowed to prehydrate. Prehydrating the splits is important as it decides the hydration rate of the final product.

7.2.1.3 Pulverization

Endosperm obtained from the screened splits is then pulverized and purified followed by grinding to obtain a powder.

7.2.1.4 Sieving

The grounded powder is sieved to obtain a desired and required mesh.

7.3 Synthesis of Guar Gum-based Therapeutics

Guar gum has been used in various therapeutic applications such as in antihypertensive drugs, anticancer, anti-infective, and anti-inflammatory drugs, which is represented in Figure 7.3.^{18,33–39} It is of particular interest due to several –OH functional groups present in long branched chains available for chemical modification. Its antioxidant and mucoadhesion properties give it the advantage to be explored for drug delivery applications in the gastrointestinal system.¹⁴

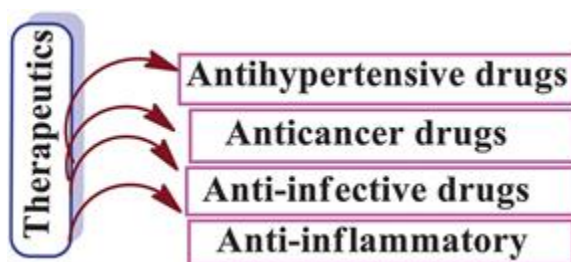


Figure 7.3 Different types of therapeutics.

Its physicochemical properties are also suitable to carry out modifications suited to different applications. Its stability over a wide pH range supports its use in GI environments over a fluctuating range of pH. Due to this advantage, it can survive degradation by microbes in an intestinal fluid environment. Another important factor is its high swelling capacity due to its hydrophilic nature. This leads to a desirable intake of aqueous medium and release of drug after modification.^{40,41} It may be tailor-made with controlled uptake of aqueous media and controlled drug release as

optimized in a particular pH environment. This would require its modification with suitable chemical methods which will design a specific grade of material suited for therapeutic applications in the GI tract.

Guar gum has been successfully grafted with methacrylic acid,³⁹ acrylamide,^{42,43} polyvinylalcohol,⁴⁴ *N*-isopropyl acrylamide,⁴⁵ and polyacrylonitrile⁴⁶ for drug release study applications. It has been employed as a matrix for dexamethasone and budesonide delivery.⁴⁷ This study concluded that the rate of release was minimal in gastrointestinal fluid and increased in colonic fluid. We have also carried out the release of 5-ASA drug using GG grafted polyacrylamide as a matrix using pH as a factor for the release phenomenon.⁴⁸ As we expected, the release was slow in acidic pH simulating the stomach environment while it was greater in a neutral and alkaline medium simulating the colonic release site. Another study reported clinical trials for mebendazole release as a comparative assessment between GG matrix tablet and immediate-release tablet.⁴⁹ It was found that GG-based colon-targeted tablets released the drug 100% into the colon and not in the stomach or other regions. Similarly, GG-grafted-with poly(ϵ -caprolactone) was utilized for the release of ketoprofen. It reported more than 200 percent grafting in resultant grades and demonstrated a sustained release over 10–68 hours.^{50,51} Therefore, it is quite clear that guar gum has been a popular choice among researchers for therapeutic applications. It is indeed one of the best available materials to work in a drug encapsulation matrix for colon-specific targeted delivery and other applications.

7.3.1 Antihypertensive Drugs

Under the class of therapeutics, the drugs encapsulated using GG are verapamil hydrochloride, metoprolol tartrate (MT), trimetazidine dihydrochloride (TMD), diltiazem hydrochloride (DIL), metoprolol succinate (MS), nifedipine (NFD), and propranolol hydrochloride (PPL).⁵²

It is well known that these drugs have short release periods which often lead to rapid release up to 3 or 4 hours. Therefore, an attempt to prolong the release times of these medications has been tried using guar gum as a matrix for oral delivery. This needs to be studied scientifically, from *in vitro* studies to *in vivo* data, clinical trials, and finally availability of

commercialized products based on standard protocols and necessary approvals.

Most of the drugs derived from guar gum can broadly be categorized into solubility of water. Some of the drugs also block the calcium through their hepatic metabolism and a half-life of 3–4 h with the anti-hypertensive effect of a few hours. Also, the bioavailability of these water-soluble drugs is often in question due to very low reported bioavailability (10–20%) upon oral administration. It undergoes a rapid transformation in the liver with a half-life of 4 h. Similarly, a calcium ion channel blocker has a plasma elimination of 3–4.5 h. Several efforts have been made to improve these drug release rates.^{53,54}

7.3.2 Anticancer Drugs

Of the widely used drugs for cancer treatment, 5-fluorouracil (5-FU), curcumin, and methotrexate (MTX) are reported using GG as their polymeric matrix. Here, 5-FU has been studied for its efficiency in the treatment of colorectal cancer. However, 5-FU administered in the form of injections seems to have some toxic effects. Hence, the CR 5-FU drug delivery was tried in the colon by reducing its side effects. This could be achieved by combining its duration of treatment with its dosage.

To achieve this safe delivery, Sinha *et al.*^{55,56} synthesized instant dispersion producing tablets that had 5-FU incorporated in a xanthan and guar gum matrix which gave 40% drug release in 24 h. Drug production was increased to 80% where there is cecal content. Later on, matrix tablets containing 5-FU^{57,58} along with only a GG matrix released 3 to 4% of the drug in simulated GI fluid and up to 70% in the simulated colonic fluid. Ji *et al.* synthesized 5-FU tablets through a fluidized bed coater system. In this system, an aqueous/ethanol mixture of GG and ethyl acetate (EA) was sprayed to make up for the drug release delay by trying GG : EA ratios in various combinations for colon delivery. For example, at the 2 : 7 ratio, there was no disintegration up to 5 h and it attained a slow-release up to 90% in 16 h.

Nowadays many formulations have been tried in the form of microspheres. GG-based microspheres are also reported which comprise 5-FU⁵⁹ along with a crosslinker for colon targeted delivery. Cytotoxicity

studies were carried out for these using human HT-29 colon cancer cell lines and it gave 99.99% cell viability concerning 5-FU release for 24 h giving signs of rapid apoptosis. GG microspheres containing 5-FU⁶⁰ synthesized by an emulsification process were also reported with 15% of drug release in gastric juice in 5 h. However, the same composition led to the release of 90% of the drug in the case of 4% rat cecal during four days of enzyme insertion, which indicates that these formulations can be optimized further for specifically targeting colorectal cancer.

7.3.3 Anti-infective Drugs

Anti-infective drugs are generally used to make oral controlled-release formulation, and metronidazole (MTZ) was extensively tested. MTZ is effective in treating chronic periodontitis which is a common disease of the oral cavity. The drug was encapsulated into a graft copolymer for attachment of methacrylic acid and GG⁶¹ and its delivery to the colon was studied using the Eudragit-L100 as an enteric cover material which protected the drug in a low pH environment. In the stomach media (for 2 h) and intestinal fluid at pH 7.4, about 70% of the drug was released, and the structure formed issued only 18–24% of MTZ. More research on MTZ loaded multilayers and compressed tablets containing various compositions of GG⁶² showed 43–52% as well 25–44% of release, respectively, in the stomach and intestines, depending on the structure of the GG. These tablets released <1% of the drug in the stomach and intestines before 5 h, and in moderate colonic fluid, 61% was released after being damaged by colonic bacteria for 24 h.⁶³

7.3.4 Anti-inflammatory

Flurbiprofen is a class of NSAID used in the treatment of rheumatoid arthritis, osteoarthritis, and similar other ailments for a drug release profile with a half-life of 4 h. To improve CR dosage forms, wet granulation prepared GG matrix tablets containing Flurbiprofen⁶⁴ and this showed a 97% *in vitro* release in a phosphate buffer of pH 7.4 for at least 12 h. Similarly, Indomethacin is another drug in the same category prepared as

tablets⁶⁵ that use a XG and GG mixture with different compositions where there is cecal content of rats to control the release in the upper GI tract.

Aceclofenac (ACF) is a common drug used for pain relief of all kinds, inflammation due to various causes, and similar other symptoms. This drug works on the mechanism based on cytokine inhibition, therefore it has a higher anti-inflammatory activity than any other NSAID. Another study has shown that compression-coated tablets⁶⁶ containing a drug (Aceclofenac) using various GG and hydroxyl propyl methylcellulose have been shown to release 6% of the drug in the stomach as well as in the intestines for 6 h because after that 30% release occurs without the content of cecal rat. Before rat content, 92% of the drug was released in the colon. Another compressed tablet containing ACF drug prepared using⁵² formulations of GG and locus bean gum with XG as a cover material where 5% of the drug was released in the first 5 hours and at the end 33–53% released at 24 h. In a recent study,⁶⁷ the tablet matrix made of using EC and GG designed in such a way for oral CR formulations using ACF stable drugs that no changes occurred in physical appearance even after being stored at 40 °C for six months.

7.4 Different Ways of Giving Drugs Using a Polymer Blend

7.4.1 Particulate Level Blends

Particulate level blends require mixing polymer powders of comparable size together which retains their sizes as such and they do not undergo any further size change. Drug procedures follow specific techniques such as compression, granulation, rolling, and compaction, *etc.* which have a particulate size of tens of hundreds of microns. Their interaction is generally limited to surfaces only when these two distinct powder types come in contact with each other. Therefore, the polymer mixtures used for tablet formation undergo inter-polymer bonding at the particle level. Further mixing in these combined polymeric particulate blends is limited to the individual particle level only which are in contact with each other and do not show any growth mechanism. Some localized mixing of particles can

also be achieved by adding water or organic solvents by wet granulation processes.^{68,69}

7.4.2 Colloidal Level Blends

In a colloidal level mixture type, at least one of the blended polymers mainly exists in surface conjunction or is in the range of submicron size. These kinds of blends can be achieved by mixing colloids (*e.g.*, latex polymer dispersion) in various proportions, phase separation (*e.g.*, from solution, dispersion, or dissolution by processed polymer compounds). Polymeric micelles are also mixed to yield colloidal level blends. *E.g.*, two or more amphiphilic assembly block copolymers⁶⁷ may form this class of polymer blends. An increased surface area leads to the creation of better interpolymer interactions in these blends as compared to particulate level mixing compositions. The surface charge also plays a vital role in these polymer blends.

7.4.3 The Molecular-level Blends

Molecular mixtures are created to bring about interactions at molecular levels. This may be done by using common solvent processing or subjecting these blends to thermal processing by melting in or above the glass temperature (T_g) ranges. Then, the thermodynamic compatibility, temperature, blend ratio, and polymer composition can result in detectable phases. Homogenous polymers created at the molecular level comprise polymeric films, capsules, gels, and solutions. Polymer–polymer miscibility in all components, hypromellose (HPMC) has been identified as well as methylcellulose mixture films, with the following good mixing behavior.⁷⁰ Polymer miscibility is desirable in films and capsule shells because it generally results in clear and transparent films. In the case of amorphous solid dispersions, the molecular scale interactions are important to prevent the crystallization of the drug during its shelf life. Thus the polymer–polymer miscibility is important to reduce the phase separation.

7.4.4 Excipients

Excipients are one of the key components in drug release applications. Commercially available Avicel® CE 15 is also a polymer blend excipient, formed by microcrystalline cellulose combined with guar gum (85 : 15) powder. It provides a good oral solid dosage in the form of chewable tablets. The combination of microcrystalline cellulose and guar gum has also been studied as a substitute for fat due to their favorable characteristics.⁷¹ This combination product gives a creamy taste to give an overall feel of food fats to be used in chewable tablets.^{72,73} Avicel® CE 15 is made by a co-processing technique involving a microcrystalline cellulose and guar gum mixture into circular shapes by spray drying.⁷⁴ Its commercial production uses MCC as the starting material, which can be made into a colloidal level blend. Beneficial organoleptic properties of the Avicel® CE 15 are the result due to close interactions during co-processing and the round shape of the aggregates.⁷⁴

7.4.5 Film Coatings and Oral Films

Polymer mixtures are widely used as film coatings for tablets, pellets, and capsules. A second polymer can be used with the primary film to enhance the adhesion to the substrate, change the equipment dressing properties, and improve the anhydrous properties. Cost reduction could be another reason to make polymer blends. Several commercial film marketing programs are based on polymer composites. These include both non-functional (immediate-release) and functional (controlled release) coatings.

The purpose of non-functional coatings is mainly due to esthetic feel, product identification, stability toward light and moisture, and taste masking. For non-functional coating, the low viscosity HPMCs are the most commonly used polymers. To use HPMC⁷⁵ for tablet coating, the plasticizer *i.e.* polyethylene glycol (PEG) is required to improve film flexibility. Another reason for using polymeric blends in non-functional film coatings is for the better stability of drugs by lowering water vapor permeability, time utilization of the coating process, and to produce dry, fully formulating coating systems. Functional film coatings have been extensively used as controlled drug release applications.⁷⁶

7.4.6 Tablets

While tablets are composed of a complex mixture, their non-polymeric excipients such as sugars and polyols are possibly the most common ingredients. Based on the overall weight of the tablet, certain polymer blend compositions give different efficiencies. In terms of direct compression and liquid-based powders, these products can be categorized as particulate level blends without the prior discussed co-processing in collaboration with Avicel® CE 15 excipient.

Polymers are widely used in the preparation of continuous release tablets. The sustained-release products are beneficial as they provide improved control of the treatment window. Matrix tablets based on polymers form a hydrogel commonly used in this case.⁷⁷ Gel forming builds a diffusion barrier to water and soluble drug substances. Gel erosion also contributes to drug withdrawal, especially in cases of insolvency.

7.4.7 Capsules

Gelatin was the only option for many years to build a solid and soft capsules formula. However, the origin of its animals, bovine spongiform encephalopathy (BSE) concerns, and its ability to cross-link have been major problems that led to the creation of non-animal origin capsules. Many of these plant-based capsules use more than one polymer to form the capsule shell.

Another application of polymer blends has been in the development of non-coated enteric hard capsules. While film-coated capsules were coated with enteric polymeric, this process adds additional unit performance and level of the difficulty of making a drug product. The progress of capsules with integrated input function inside the shell is, therefore, a remarkable asset in capsule technology. Another benefit of enteric capsules is that they can be used in very few batches that are prepared by filling individual capsules by hand or with machines to fill small capsules which are very helpful in the growth of early drugs as well preclinical studies where there is limited availability of drug substance.

Nowadays soft capsules derived from plant origin are also available which are more amenable toward liquid, paste, and oil fills than hard capsules. Immediate release of non-gelatin soft capsules that are made of modified starch and carrageen are commercially available.^{68,78,79}

7.5 Applications

Currently, natural polysaccharides and their derivatives are widely used in pharmaceutical formulations, particularly for controlled drug release studies. Guar gum is often preferred as a binding and disintegrating agent in tablet formulations. In liquid formulations also, it is used as a thickener, emulsifier, stabilizer, or suspension agent. Moreover, its properties can be further modified to attain the desired solubility, swelling, film-forming, and other characteristics. Hence, in recent years guar gum has been intensely explored toward therapeutic application including targeted and controlled release studies in all kinds of formulations.

Therefore, guar gum has emerged as one of the popular choices among researchers for its applications as is and after any kind of modifications. It has inherent flexibility which suits the drug release profile, is low in cost, is widely available, and is hence accepted for the targeted drug delivery by various modes including oral drug release systems, as coatings, matrix tablets, hydrogels, as well as nanoparticles.

7.5.1 Guar Gum-based Microparticles for Drug Delivery

Recently, Gowda *et al.* prepared GG-based microparticles with the 5-fluorouracil (5-FU) drug for colon targeted delivery.⁸⁰ These guar gum drug-loaded microparticles were prepared using the emulsification technique by trisodium trimetaphosphate (STMP) as a binder/linker. Surface morphology studies using scanning electron microscopy (SEM) show that these microparticles are present as nanoaggregates with a spherical surface. Better drug release results were obtained from enzymes from rat cecal content analysis as compared to intestinal fluid.

Chaurasia *et al.*⁸¹ studied GG-based drug delivery by preparing GG microparticles for methotrexate release. These microparticles were prepared by crosslinking with glutaraldehyde (GA). Various factors were considered before and during the preparation of these microparticles and it was found that surface morphology features of microparticles like their shape, size, *etc.* were influenced by the concentration of both guar gum and glutaraldehyde, span (80), temperature, rate, and stirring time. They were tested for *in vitro* drug release from various mediums, like phosphate buffer,

gastrointestinal fluids at different pH, and for rat cecal content.⁸² As expected, it was clear that *in vitro* drug release was influenced by the GG and GA concentrations. It also showed methotrexate (91%) content in rat cecal content with buffer. The results could be correlated with the *in vivo* studies where GG MPs released maximum methotrexate (79%) in the colonial region, while methotrexate suspension released 23% of its total dosage to the targeted sites.⁸¹

7.5.2 Guar Gum-based Nanoparticles for Drug Delivery

Some researchers have been trying to develop an effective drug release mechanism for tuberculosis. It has been found challenging that the existing BCG vaccine is not sufficient for adult pulmonary tuberculosis and preparing a drug release mechanism with guar gum-based nanoparticles and nanocomposites has been investigated.^{83–85} This was done by strengthening the immune response of the body against foreign invasion. Kaur *et al.* prepared GG NP Santigen (Ag85A) for oral delivery against tuberculosis.⁸⁶ Nanoprecipitation is a technique for the formation of nanoparticles with a particle size of approximately 895 nm. This was confirmed by various tests like the acid test, Peyer's patch analysis using confocal microscopic study, and *in vitro* antigen analysis. Further investigation into the simulated intestinal fluid (pH 7.2) showed that the prepared dosage form was sufficient to protect antigens from the acidic medium and this could allow the antigen to pass into the intestinal tract. *In vivo* studies using the mice model also showed that modified NPs were able to have a systemic immune response resulting in a strong mucosa.⁸⁶

These studies were extended toward the development of guar gum nano aggregate particles prepared by GG through the precipitation technique for the delivery of tuberculosis drugs like rifampicin and isoniazid.⁸⁷ These nanoaggregates were evaluated in terms of density, morphology, drug entrapping efficiency, and corresponding *in vitro* release. These GG nanoparticles showed a unique drug release profile through the lungs. Furthermore, Dutta *et al.*⁸⁸ synthesized nanocellulose and copolymer-based nanocomposites. They contained guar gum in various combinations with poly(*N*-isopropyl acrylamide) (PNIPAAm) and the compound was confirmed by NMR and FTIR characterization studies. Nanocomposites

were formulated to add nanofibers (0.5×10^2 wt%) to GG-graft-PNIPAAm. The nanocomposite contained nanofibers in the form of patches which showed non-irritant and cytocompatibility behavior *in vitro*.

7.5.3 Guar Gum-based Nanoparticles as Self-healing, Injectable, and Antibacterial Biomaterials

Biomaterial-based hydrogels can be fabricated so that they have antibacterial agents. These can provide stable solutions for the failure of biomedical devices and disease prevention. Guar gum cross-linked with borax-containing silver nanoparticles showed rapid self-healing properties. This could also be used by injecting the material into biomedical devices. It also exhibits antibacterial properties. These hydrogels are characterized⁸⁹ by various techniques like infrared spectroscopy, thermogravimetric analysis (TGA), scanning electron microscopy (SEM), and rheological measurements.⁹⁰

7.5.4 Self-healing Properties

Guar gum-based hydrogels show excellent self-healing properties. Some case studies show that guar gum-based hydrogels have one of the best self-healing properties among their other counterparts.^{91–93} Some studies were conducted for this interpretation; hydrogels were divided into two parts and one of them was dyed with Rhodamine 6G to have complete visualization of biological properties. For this, two identical hydrogel pieces were cut apart and later left in contact with each other to test their self-healing properties. They were left in ambient conditions without a change in temperature or light/pressure/chemical stimuli. Once the evaluation period was over, the test results showed that each weight% of borax guar gum hydrogel indicated appreciable self-healing within 6 minutes. The rheological properties showed that the test healing gels were completely recovered under pressure. These results showed good gelling ability leading to the formation of durable borax compounds.⁹⁴

7.5.5 Injectable and Antibacterial Properties

In an attempt to develop potential biomedical materials, guar gum-based hydrogels were incorporated with silver nanoparticles to provide antibacterial properties and to enhance the injectability potential of the gels. In these kinds of compositions, the ratio of GG : borax in the hydrogel was around 5 : 1 which had a low borax concentration.⁹⁵ This helped to bind the hydrogel as a crosslinker and to retain a stable gel structure. This is an important quality for the injectability purpose of hydrogels to withstand deformity. So, based on their suitability to be used as injectable gels they could be used as liquids, and gel formation took place after injection.⁹⁶

The GG/Cur-AgNPs hydrogel reveals strong antimicrobial activity against antibacterial properties like Gram-negative bacteria, most common *E. coli* and *P. aeruginosa*, as well as Gram-positive bacteria⁹⁰ like *S. Aureus* forming inhibition zones of various diameters like 13.5, 13.0, and 15.0 mm respectively. Also, these hydrogels containing nanoparticles could be used in syringes because of their anti-microbial, biodegradable nature, and could be applied for injectable formulations fit for biomedical and industrial applications.^{97,98}

7.5.6 Guar Gum-based Grafted Systems for Drug Delivery

Tiwari and Prabakaran have reported the synthesis and applications of GG-based nanocarriers for the delivery of ketoprofen⁹⁹ using NSAIDs as a hydrophobic model drug.⁵⁰ Here, amphiphilic GG was grafted to poly(ϵ -caprolactone)(PCL) using microwave irradiation to yield GG-grafted PCL copolymers. A GG-graft-PCL graft composite has been used for the development of spherical-shaped micelles. These micelles are of nano-size and are intended to be used in a wet medium. The diameter of these nanocarriers was determined using the DLS technique, which showed the average diameter in the range of 75–135 nm. The *in vitro* drug release profile was tested at a temperature of 37 °C and pH 7.4. GG-graft-PCL-loaded drug micelles showed the burst pattern in the first phase followed by an extended drug release.

Soppimath *et al.* reported microparticles composed of poly(acrylamide)-grafted-GG [PAM-graft-GG] hydrogels. It had glutaraldehyde as a linking agent to control the release of verapamil HCl and Nifedipine¹⁰⁰ drugs. The kinetics of swelling studies show that a fluid change shift takes place from

the Fickian pattern to the non-Fickian pattern as the composite material concentration was increased during the preparation. *In vitro* release of the drug, compounds showed a correlation between the number and modes of drug loading, nature of the drug, and other related factors. The hydrogel microparticles showed a good swelling and drug release pattern.

7.5.7 Guar Gum-based Hydrogel Systems for Drug Delivery

Polymer-based hydrogels are found to be important in the development of controlled releasing drug delivery to a targeted site due to their significant properties.¹⁰¹ In research, Bajpai and Sharma (2006) reported pH-dependent water uptake of barium alginate/carboxymethyl guar gum hydrogel beads. The resulting hydrogel bead swelling behavior with varying pH from simulating gastric fluid (SGF) to simulated intestinal fluid (SIF) shows enhancement. The treatment results showed that the drug releases closely w20% in SGF after 3 h and closely w70% in SIF at 7 h.¹⁰²

In one of the studies, George and Abraham reported a pH that responds to alginate–GG hydrogels with a controlled way of protein delivery.¹⁰³ GG–alginate hydrogel crosslinking shows the high concentration of a drug protein, BSA tested in two different fluids, SGF at pH 1.2, and SIF at pH 7.4. Hydrogels loaded with BSA alginate to GG of 3 : 1 ratio have shown effective efficacy of the drug in these polymeric particle hydrogels. The BSA release pattern from alginate–GG hydrogel beads was found to be w20% in SGF (acidic pH) and was found to be notable w90% in SIF (modest alkaline pH). *In vitro* studies have clearly shown that the incorporation of GG and GA cross-linker has gained the efficiency of protein synthesis. This has also preserved the BSA from being released into the acidic environment and has shown an alkaline controlled release in the intestinal pattern.¹⁰³

7.5.8 Guar Gum-based Buccal Film for Drug Delivery

Oral delivery of a variety of protein and peptide-based drugs remains a rigorous task for researchers studying drug delivery due to the low availability caused by low exposure to absorption epithelia, metabolism, and degradation. Aiming to overcome limitations, Castro *et al.* introduced nanoparticles on peptide-filled poly(lactic-co-glycolic) acid within the GG

matrix of the film.¹⁰⁴ All these formulations were investigated using the cell line of buccal carcinoma with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for the experimental reduction. The introduction of poly(lactic-co-glycolic) nanoparticles with GG films can show increased permeability through buccal and suitable carriers for antihypertensive peptides.

7.5.9 Guar Gum-based Tablets for Drug Delivery

Sinha and Kumria prepared matrix-loaded indomethacin tablets to constitute GG and xanthan gum (XG) in varying doses⁶⁵ and formed matrix tablets showing drug release in the upper GIT as well as in the chemical digestion process. In another study, Sinha *et al.* reported a GG-based tablet containing 5-FlouroUracil used for cancer treatment.⁵⁵ An *in vitro* drug release by XG : GG (10 : 20) showed a 5-FlouroUracil increase in colonic fluid content, where w67% in the occurrence of 2% cecal content and w80% in the occurrence of 4% cecal content occurs after a 19 h incubation period.

In vivo pharmacokinetics and *in vitro* dissolution were observed using the high-performance chromatography (HPLC) method. The *in vitro* dissolution pattern¹⁰⁵ showed the release of w4% ketorolac tromethamine in 5 h and further expansion drug release w99% in 24 h. *In vivo* pharmacokinetic tests have shown the high plasma concentration ($C_{\max}$) value of immediate-release tablets was 4524.61 ng mL⁻¹ with $T_{\max}$ of 2 h and $C_{\max}$ of 3396.83 ng mL⁻¹ with $T_{\max}$ of 10 h for colon-specific tablets. The lower plasma concentration of ketorolac tromethamine compared to the period of rapid drug released curves. These GG-based compounds showed 10894.67 ng h mL⁻¹ and 17647.58 ng h mL⁻¹, respectively, with a mean residence time of 3.92 and 10.25 h. Tuğcu-Demiröz *et al.* investigate mesalazine (5-ASA) loaded GG-based matrix tablets for colon-specific drug release. *In vitro* and *in vivo* X-ray studies of 5-ASA tablets-GG-based matrix tablets were performed. The high-viscosity GG-based matrix tablet has been shown to block the drug released from the upper GIT. Investigating X-ray imaging experiments to locate the matrix tablets in healthy volunteers, where barium sulfate was used in X-ray imaging in matrix tablets *in vivo* research.^{105,106}

Krishnaiah *et al.* observed a GG-based tablet in healthy male volunteers using a non-invasive technique gamma scintigraphy tests in front of the tracer technetium 99m-DTPA.^{107,108} After testing the result showed that a small tracer was present in the surface of the tablet, which was released in the acidic area as well as in the small intestines. But most of the tracer was released to the colon site within 2–4 h in the delivery time of the tablet. The GG-based hydrogel contains trimetazidine dihydrochloride, an antihypertensive drug for oral delivery administered by Krishnaiah *et al.*¹⁰⁹

7.6 Guar Gum-based Microsphere for Delivery of Anticancer Drugs

Guar gum is hydrophilic, in cold water guar gum swells, able to form viscous colloidal dispersions. Guar gum was found to be suitable for colon-specific drug carriers in the form of a matrix, tablets, as well as microspheres. Guar gum-based microspheres were prepared using an emulsification method in which glutaraldehyde was used as a crosslinking agent. The concentration of guar gum and span 80 (emulsifier) affect the particle size, the shape of the microsphere formed, and surface morphology. The drug–polymer interaction was determined using the dialysis technique. In this technique, MTX (methotrexate) was dissolved in phosphate-buffered saline and the polymer solution was dissolved in drug solution with continuous stirring for 1.5 hours.⁸¹

7.7 Toxicity Status of Guar Gum-based Therapeutics

Guar gum (GG) is a naturally occurring, non-toxic polysaccharide and is a cheap material that protects the bioactive molecule from being completely released into the body of the stomach and small intestine, but removes the molecule from the colon.

7.8 Alternatives to Guar Gum

Based on the composition, structure, chemistry, and suitability in various applications, we can conclude that guar gum is undoubtedly the most researched and widely accepted material to be used in therapeutic applications. Still, if we consider its future, it is expected to be available in plenty due to the conducive conditions of growth, processing, established market, and its acceptance among researchers, and huge commercial potential. It is undoubtedly going to continue in its position as the prime contributor to the overall share of gums concerning market capture. Even then, alternatives to guar gum may be developed for therapeutic application and other applications also. These gums are those which have similar characteristics and tendencies, modification potential, wide availability in localized regions, and non-toxic nature. Some of these gums may be gum ghatti, Jhingan, tragacanth, moringa, and other gums which may be chosen depending upon their availability and cost to reduce the burden on guar gum for all the applications.

7.9 Conclusion

Based on the above review, it may be concluded that guar gum has emerged as the most suitable material for therapeutic applications. Its low cost, wide availability, nontoxic nature, food-grade status, hydrophilicity, and easily modifiable structure make it a good material with huge commercialization potential. Recent patents and products also indicate that guar gum has a huge market for the therapeutic products derived from it.

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Chapter 8

Gellan Gum-based Drug Delivery Carriers

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8.1 Introduction

Recently, world-wide attention has been being paid to environment-friendly green manufacturing of various pharmaceutical and biomedical products with biodegradable and biocompatible ingredients. In this context, natural biopolysaccharides are attracting the attention of drug delivery and biomedical researchers for their physiological compatibility and biodegradability.¹ Easy availability, low cost, hydrophilicity, chemical inertness, and physical and chemical compatibility with a wide range of bioactive compounds also make them feasible to be used as pharmaceutical, food, and biomedical additives.² Exo-polysaccharides obtained from bacterial sources have been considered as potential and economically competitive with others obtained from plants, marine, or algal sources. Classical examples of such bacterial exopolysaccharides are gellan gum produced by *Sphingomonas paucimobilis*, xanthan gum produced by *Xanthomonas campestris*, and dextran produced by *Leuconostoc mesenteroides*.³ Gellan gum and its different chemical derivatives have been established as hydrogel matrices for drug delivery, tissue engineering, and other biomedical applications.^{4,5} Gellan gum has emerged as a useful additive in chemical, food, pharmaceutical, and cosmetic industries because of its unique favorable physicochemical properties in terms of practicality and processability.^{6,7} Like other biopolysaccharides, gellan is also easily available, eco-friendly and biodegradable.^{8,9} Low-cost biomaterials are fabricated with gellan gum with a high reproducible quality on an industrial scale.¹⁰ It has been widely used as a viscosity builder, stabilizer, suspending agent, emulsifying agent, gelling agent, tablet diluent, binder, lubricant, film former, and coagulant.^{7,11} This chapter presents various sources and production of gellan gum along with its chemical structure, molecular features, different grades, and their different physicochemical properties. Brief descriptions of various chemical derivatives of gellan and their purposes have also been presented. Different drug delivery systems fabricated with gellan gum or its chemical derivatives along with their applications have also been discussed.

8.2 Sources and Production of Gellan Gum

Gellan gum is a widely explored bacterial exopolysaccharide synthesized by the bacterium *Sphingomonas elodea* belonging to the family of sphingans.^{1,11} These bacteria were isolated from elodea plant tissue.¹² But, in 1994, another bacterium *Sphingomonas paucimobilis* was discovered to be a producer of gellan gum and categorized in the α -4 subclass of the proteo bacteria.¹³ *Sphingomonas*

represents a group of rod-shaped, Gram-negative, chemoheterotrophic, and aerobic bacteria which contain glycosphingolipids as their cell-wall components and are found to form yellow-colored colonies.¹⁴ Gellan gum was discovered in 1978 by the Kelco, a former division of Merck & Company, Inc.¹⁵ The gellan gum producing bacterium *Sphingomonas paucimobilis* is a Gram-negative, non-pathogenic, non-fermentative, aerobic, and non-spore-forming bacillus capable of aerobically synthesizing the extracellular-polysaccharide gellan gum and the ATCC 31461 strain of this bacterium is mainly used for gellan gum production in industry.¹² The culture media used in the small or large-scale production of different bacterial exopolysaccharides plays an important role and influences the rate of polymer biosynthesis, total yields, and also the molar mass of the polysaccharides.¹

8.2.1 Factors Affecting Gellan Gum Production

The factors affecting the production of gellan gum include media components, carbon source, nitrogen source, addition of precursors, pH, agitation rate, dissolved oxygen and oxygen transfer capacity, and temperature. The culture or growth media used in the production of gellan gum to support bacterial growth are composed of different nitrogen sources, carbon sources, and inorganic salts. An ample diffusion of exopolysaccharides is found when the bacterial culture is provided with an adequate amount of carbon source and a minimum amount of nitrogen source.¹⁶ Monosaccharides such as sucrose, glucose, fructose, lactose, and mannitol are used either individually or in combination as the required carbon source. The 2–4% w/w of carbon source is usually used.¹² After carbon, nitrogen is the second most important nutrient for the promotion of the growth of the bacteria. Additionally, the type of nitrogen source along with its concentration in growth medium affect the carbon flow inside the media as well as yield of the product.¹ In various studies, the maximum yield was found with minimal nitrogen. The selection of the nitrogen source also has a strong influence on the characteristics of gellan broth. Dreveton and co-researchers reported the accelerating effect of organic nitrogen on the cell growth and biosynthetic production of gellan gum.¹⁷ The incorporation of precursor molecules is also very important in the bio-production of bacterial polysaccharides in order to enhance or stimulate the metabolic process. For example, higher concentrations of nucleotide phosphate sugars inside the cells under a restricted nitrogen-environment were reported to augment metabolite-flux in the biosynthesis of exo-polysaccharides.¹⁸ The pH also plays a very significant role in gellan gum production by *S. paucimobilis*, as it affects both cell growth and yield significantly. The optimum pH for the production of bacterial polysaccharides is found to be higher than that of the production of fungal glucans. The pH for the production of gellan gum should be within the range from 6.5 to 7.^{17,19} More acidic or more alkaline pH is found to reduce the cell replication and subsequent gellan gum production.²⁰

Optimum agitation of the culture mass is required to promote the production of bacterial polysaccharides. Agitation at 250 rpm with a helical-shaped ribbon impeller is optimum for uniform mixing of the broth. Agitation even at a low level was found to be adequate for uniform mixing. It has been reported that stirring at higher speeds (600–800 rpm) with Rushton turbine results in the formation of cavities in an impeller followed by formation of a stagnant layer because of the high pseudoplastic property of the broth. Subsequently, the broth becomes heterogenous at higher stirring speeds, which might limit both heat as well as mass transfer.¹⁷ Oxygen has been proposed as a very important component for the production of bacterial exopolysaccharides such as gellan by Rho and Loung.²¹ Oxygen deficiency in the broth reduces the growth of the bacteria and therefore the production of gellan gum. The dissolved oxygen tension (DOT) level promotes the rate of oxygen uptake by the bacterial cells, which results in a higher production of gellan. The study showed that higher DOT levels result in the production of gellan with higher molar mass and viscosity.¹ The temperature for the production of gellan gum is mostly maintained within the range from 20 to 30 °C.²² It has been reported that the yield

of the gellan gum attains the highest level at 20 °C and this level remains up to 25 °C and radically reduces above 30 °C.²³

8.2.2 Isolation of Gellan Gum from the Culture Broth

Kang, Veeder, and Kaneko described the isolation of gellan from its culture broth. The culture broth is heated to 90–95 °C for a period of 10–15 min to kill the bacterial cells, reduce the viscosity of the broth, and thereby facilitate mixing at the time of precipitation. The gum polysaccharide is isolated from the bacterial cells and broth by means of either centrifugation or filtration. The supernatant is mixed with isopropyl alcohol (ice-cold) and the resultant mixture is maintained at a temperature of 4 °C for a period of 12 h in order to complete the precipitation of the gum. The gum is finally collected after centrifugation and dried at 55 °C for 1 h.¹²

8.2.3 Purification of Gellan Gum

The gellan gum polysaccharide isolated using an alcoholic precipitation method is thoroughly washed with first acetone and then ether. Then it is dispersed in de-ionized water followed by dialysis against de-ionized water with a dialysis-membrane with a molar-mass cut-off of 12 000–14 000. The dialysis is continued for two to three days and finally freeze-dried to obtain dry gum.²² Gel filtration chromatography is also used to purify the gellan gum polysaccharide.

8.3 Chemistry of Gellan Gum

Gellan gum (GG) is a negatively-charged linear chain polysaccharide. It is composed of a repeating tetrasaccharide unit [α -glucose ($\beta 1 \rightarrow 4$) α -glucuronic acid ($\beta 1 \rightarrow 4$) α -glucose ($\beta 1 \rightarrow 4$) α -rhamnose ($\alpha 1 \rightarrow 3$)]_n. The pristine gellan gum possesses an α -glyceryl substituent unit at the carbon atom at the 3-position of the α -glucose unit at the 3-position of the repeating unit and, an acetyl group in some repetitive units on the same glucose unit.^{3,9,24} Alkali treatment can remove these substituents from the repeating unit in the presence of heat.^{25–27} Gellan gum is often sold in deacetylated form.⁹ The X-ray diffractogram of the gellan gum reveals its ordered structure in solid form.²⁸ The most widely accepted model consists of a double helix with the same axis, where every chain twists thrice vertically. The individual chain crosses each other several times due to the presence of interactions between various monosaccharides of the repeating units of the polysaccharide. The first three $\beta(1 \rightarrow 4)$ glycosidic linkages of the repeating unit allow equatorial bonds, while the fourth $\alpha(1 \rightarrow 3)$ linkage forces another twist and supports the geometry of the double helix.²⁹ This polysaccharide exhibits a unique thermoreversible self-folding capacity, which offers a wide range of applications in drug delivery and the biomedical field including cell entrapment.

8.4 Physicochemical Properties of Gellan Gum

When gellan gum was first discovered it was used as an optimum substrate in the cultivation of thermophilic microorganisms due to its physical resistance.³⁰ Subsequently, this gum has been explored in several food and pharmaceutical industries, as well as biomedical applications including tissue engineering and nanomedicines, due to the unique physicochemical and biological properties of gellan gum. It is also considered as a suitable acid and heat resistant biomaterial.³¹ The fraction of repeating acetylated units in the polysaccharide backbone can greatly influence the rheological and viscoelastic behavior of gellan-based hydrogels and aqueous dispersions.³² The acetyl groups can be removed from the repeating units by heating at 80 °C for 10 min with an increase in pH up to 10 using 1 M sodium hydroxide and subsequent neutralization with 1 M HCl acid.

Homogeneous dispersion of gellan gum is obtained after a heating process at 90 °C because a random coil condition is achieved at this temperature. Gellan gum is categorized as high acyl and low acyl-gellan depending on the percentage of acylated repeating units in its polysaccharide backbone. Both high acyl and low acyl types go through a conformational change from random coil to double helical structure, while the cooling stage occurs at about 30 °C.³³ A schematic representation of the transition is presented in [Figure 8.1\(b\)](#). Microstructural deviations develop variation in the textural behavior of corresponding hydrogels. Thermoreversible hydrogels which are soft and elastic in nature can be produced from high acyl gellan, whereas brittle gels with high thermal stability can be obtained from low acyl gellan.³⁴ A mixture of low and high acyl-gellan is found to provide gels having common properties depending on their ratio in the mixture. High acyl gellan contains a glyceryl group in each repeating unit and a fractional acetyl substitution on it. Glyceryl substitution facilitates relatively more H-bond formation resulting in comparatively stable and softer gel network in respect to low-acyl gellan gum.³⁵ In general, these differences in chemical structure are responsible for the differences in the physicochemical nature of both forms of gellan gum, attributed in several cellular encapsulation and drug delivery applications.⁹

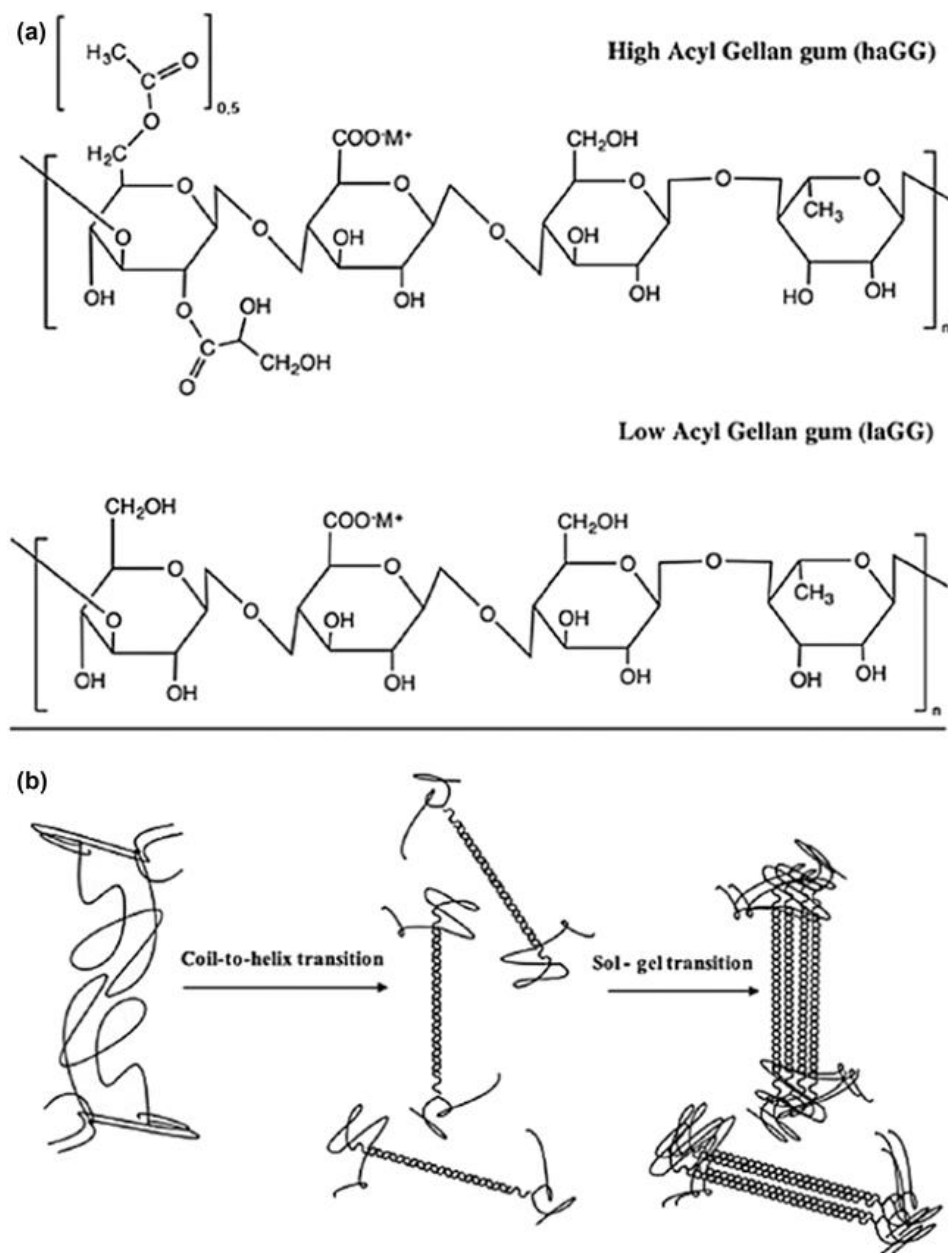


Figure 8.1 (a) Structure of high-acyl and low-acyl gellan gum, (b) coil-to-helix and sol-gel transition of gellan gum.⁹ Adapted from ref. 9 with permission from Elsevier, Copyright 2020.

One of the most important properties of native gellan gum is thermally reversible hydrogel formation. A fully homogeneous dispersion of gellan gum is obtained only at 90 °C. Free and random native gellan coils with their complex structures, start interacting as the temperature decreases. This process starts with the development of numerous double helical conformations, which result in cluster forming junction areas and the formation of a 3-dimensional network as shown in Figure 8.1(b). Additionally, neighboring carboxyl groups aid in the gelation process by complex formation with cations imparting the required strength to the hydrogel architecture. The formulation of hydrogels of gellan gum with other polymeric components may improve the physical property of hydrogel formulations for drug delivery applications.

The transition from coil to double helical structure in an aqueous dispersion of gellan gum is greatly affected by some other factors such as pH, concentration and molar mass of gellan gum, and the valence and concentration of the cations present.^{1,36} Acid solutions or monovalent salt solutions including the ammonium ions, are found to reduce the repulsion between different gellan helices, whereas divalent cations such as Ca^{++} , Zn^{++} , and Al^{+++} can bind covalently to the carboxyl groups of different chains resulting in a cross-linked gel matrix.³³ The gellan gum based 3-D gel-network demonstrates better stability in the presence of divalent cations than monovalent cations. The microenvironment acidic pH can also promote aggregation of the helical structure.³⁷ The hydrogels obtained from different types of gellan gum are found to differ in microstructures, textures, mechanical properties, and water absorbing capacity.⁹

8.5 Rationale of Gellan Gum as a Drug Carrier

Gellan gum has been widely explored in different types of drug delivery systems including solid, liquid, and semi-solid dosage forms intended for oral, topical, and also even parenteral routes of administration. The main reason behind it may be its unique physicochemical properties including ionotropic gelation and thermoreversible gelation natures. Additionally, easy production, low-cost, broad-spectrum compatibility, biocompatibility, and biodegradability are the factors which make it a potential drug carrier or formulation additive. It offers a wide range of drug delivery strategies including pH-responsive, thermosensitive, *in situ* gelling, ion-triggered drug release, *etc.* It also allows green manufacturing of different dosage forms. A lack of cytotoxicity also makes it suitable for tissue engineering, cell encapsulation, and wound dressing.^{15,38}

8.6 Chemical Modifications of Gellan Gum for Benefits of Drug Delivery

Gellan gum has been chemically modified in different ways such as carboxymethylation, graft-copolymerization, oxidation, alkylation, and ionic cross-linking, *etc.* in order to achieve different intentions of specific drug delivery systems. Some glimpses of chemical modifications of gellan gum are presented in the following subsections.

8.6.1 Carboxymethylation

A carboxymethyl derivative of gellan gum has been obtained in order to improve the bioadhesive property of gellan gum for the development of mucoadhesive drug delivery systems.³⁹ The carboxymethylation was done by the reaction of gellan gum with monochloroacetic acid in the presence of sodium hydroxide. The degree of carboxymethyl substitution (DCS) of 1.18 was achieved in the study. Furthermore, carboxymethyl derivatization was found to augment the degree of crystallinity, reduce the cation-induced gelation, and increase mucoadhesiveness by 2.71-fold. The ocular tolerance study was performed using a chorioallantoic membrane collected from hen's eggs indicating the non-irritant nature. The cytotoxic analysis was performed on Vero cells through a resazurin assay which indicated the biocompatible behavior of carboxymethyl gellan gum. Metformin-loaded beads composed of calcium-cross-linked carboxymethyl gellan gum showed 100% *ex vivo* bioadhesion over 24 h and a faster drug release profile than that from gellan.

8.6.2 Graft-copolymerization

Graft-copolymerization has also been considered a useful approach for modification of biopolysaccharides in a drug delivery strategy.⁴⁰ Nandi *et al.* described the synthesis of

polymethacrylamide-*grafted*-gellan through a microwave-assisted free-radical initiating technique employing a redox-initiator, *i.e.* ceric ammonium nitrate.⁴¹ The results of acute oral toxicity and histological study suggested categorizing the copolymer as non-toxic as per OECD guidelines. This copolymer was employed to develop diclofenac sodium containing a matrix tablet, which showed sustained *in vitro* drug-release over 8 h. The same group also demonstrated another graft-copolymerization of gellan, where gellan was grafted with poly(acrylic acid) to obtain a better mucoadhesive copolymer for fabrication of extended-release gastroretentive tablets of metformin hydrochloride.⁴² The copolymer was synthesized by the same method mentioned earlier with little modification. Tablets prepared by a wet granulation method with poly(acrylic acid)-*grafted*-gellan gum, showed excellent mucoadhesion and extended drug release over 10 hours with a dissolution similarity factor (f_2) of 77.43, where USP official release profile for metformin HCl extended release tablet was taken as the reference. The release mechanism was found to be based on Case-1 Fickian diffusion.

8.6.3 Oxidation

Another report describes oxidation of gellan and its subsequent fabrication into hydrogel beads with resistant starch by ionic cross-linking.⁴³ Resveratrol was used as the model drug. Oxidization of gellan gum was performed through a 2,2,6,6-tetramethylpiperidine-1-oxyl radical-induced process and characterized by FTIR-spectroscopy and ¹³C NMR to confirm the successful introduction of carboxyl groups into the gellan gum molecules. Resveratrol was included in β -cyclodextrin as an inclusion complex and incorporated in the hydrogel bead matrix. The hydrogel beads showed a pH-responsiveness, high drug entrapment efficiency (84.95–90.73%), high stability in gastric pH, and sustained drug-release in simulated intestinal fluids.

8.6.4 Alkylation

Low molecular weight (52.6 or 96.7 kDa) gellan gum can be functionalized using octylamine, dodecylamine, and octadecylamine in combination with a coupling agent, bis(4-nitrophenyl) carbonate.⁴⁴ The thermo-rheological and ionotropic crosslinking behaviors of these alkyl-derivatives of gellan gum and their relation with alkyl chain length and degree of alkylation were examined. The results exhibited the influence of derivatization on the coil-to-helical structure gelation mechanism of gellan gum derivatives, ionic cross-linking, and mechanical behavior of calcium and cross-linked hydrogels. The study revealed that introduction of an alkyl group on the polysaccharide backbone interferes with the coil-to-helical structure change mechanism and imparts hydrophobicity to gellan hydrogels.

8.6.5 Gellan–Thioglycolic Acid Conjugate

A gellan–thioglycolic acid conjugate has been proposed to improve its mucoadhesiveness.⁴⁵ Incorporation of thiol groups in the gellan moiety can minimize calcium ion-induced gelation and enhance crystallinity to a little extent. Thiol-conjugate gellan was found to be a non-irritant and biocompatible in *ex vivo* ocular sensitivity study with a chorio-allantoic membrane of hen's egg and a biocompatibility test by resazurin assay performed on Vero cells, respectively. Metronidazole-loaded gels composed of a gellan–thioglycolic acid complex demonstrated a 1.82-fold increase in mucoadhesiveness compared to gels composed of native gellan gum and a drug-release profile with first-order and Higuchi's square-root kinetics.

8.7 Gellan Gum-based Drug Delivery Carriers

A wide range of drug delivery systems have been fabricated with either native gellan, deacetylated gellan, or chemically-modified gellan, which includes beads, microbeads, microspheres, microcapsules, pellets or micropellets, tablets, hydrogels, *in situ* gels, emulsion, transdermal patches or films, nanoparticles, *etc.* intended for peroral, topical, ophthalmic, nasal, and parenteral routes of administration. [Table 8.1](#) summarizes different drug delivery systems based on gellan gum and its derivatives. The following sections present different types of dosage forms based on gellan or its derivatives.

Table 8.1 Different gellan-based drug delivery systems.

Types of delivery systems	Compositions	Preparation methods	Applications	Ref.
Beads	Ca ⁺⁺ cross-linked hydrolyzed methacrylamide- <i>grafted</i> -gellan and tamarind seed gum composite	Grafting followed by an ionotropic gelation method	Oral extended-release of diclofenac sodium	46
	Carboxymethyl fenugreek galactomannan–gellan gum–calcium silicate composite	Ionotropic gelation method	Oral mucoadhesive beads for sustained-release of glimepiride	47
	Calcium cross-linked gellan–jackfruit seed galactomannan composite	Ionotropic gelation method	Oral mucoadhesive sustained-release of metformin HCl	48
	Calcium cross-linked alginate–pectin or alginate–gellan matrix	Ionotropic gelation method	Sustained delivery of gliclazide	49
Microparticles	Gellan–alginate	Maleic anhydride-induced	Sustained delivery of aceclofenac	50
	Nickel-crosslinked gellan	unsaturated esterification process	Encapsulation of protein	3
	Calcium-cross-linked gellan gum	Water-in-oil emulsion method	Mucoadhesive nasal delivery of almotriptan	51
	Al ⁺⁺⁺ cross-linked gellan	Emulsification-cross-linking method	Mucoadhesive microspheres	52
	Gellan gum	Ionotropic gelation method	Mucoadhesive nasal delivery of donepezil	53
	Gellan gum	Spray dried technique	hydrochloride	54

		Water-in-oil emulsion and solvent diffusion method	Encapsulation of methotrexate	
Pellets	Gellan gum and polyvinylpyrrolidone	Extrusion and spheronization technique	As a binder in the formulation of sustained-release pellets of theophylline	55
<i>In situ</i> gel	Gellan gum and hydroxypropylmethyl cellulose	Simple mixing	Nasal delivery	56
	Deacetylated gellan	<i>In situ</i> gel due to ions in lachrymal fluid	of salbutamol sulfate	57
	Gellan along with polysorbate as solubilizing agent	<i>In situ</i> gel due to ions in lachrymal fluid	Sustained-release <i>in situ</i> ophthalmic gel for ketotifen	58
	Gellan, HPMC K100M	Raft forming <i>in situ</i> intragastric gel	delivery	59
	Aminated gellan gum	<i>In situ</i> nasal gel	Sustained ophthalmic delivery of estradiol	60
			Stomach specific controlled delivery of itopride HCl	
			Enhancement in mucoadhesion for nasal mucoadhesive preparations	
Tablets	Gellan gum and gelatin	Wet granulation method	Evaluation of swelling kinetic	61
	Gellan gum and lactose	Wet granulation method	and	62
	Gellan gum	Direct compression	disintegrating properties of gellan gum in metronidazole tablets	63
	Polymethacrylamide-grafted-gellan gum and tamarind seed gum composite	Graft copolymerization followed by tablet preparation by a wet granulation technique	Tablet binder	64
			Gastroretentive delivery of cilnidipine for enhancement of	

			oral bioavailability Gastroretentive delivery of metformin hydrochloride	
Hydrogels	Gellan gum Oxidized gellan gum, carboxymethyl chitosan Gellan–chitosan– polyethylene glycol Poly(1-lactide- <i>co</i> - glycolide) nanoparticles dispersed in calcium cross-linked gellan hydrogel	Solvent casting ionotropic gelation method Microspheres interspersed in gellan based hydrogel Polyelectrolyte complexation PLGA- nanoparticle formation by a double- emulsification method; gellan hydrogel formation by ionic cross- linking	Delivery of ofloxacin and tea tree oil for wound healing Wound regeneration Apigenin delivery in wound Local injectable preparation for vancomycin delivery in osteomyelitis	65 66 67 68
Micelles	Alkylated gellan with C ₁₆ alkyl groups Alkylated gellan with C ₁₈ alkyl groups	Self-assembled micelles Self-assembled micelles	Improvement of aqueous solubility of poorly water- soluble drugs like simvastatin Controlled delivery of budesonide from nanomicelles for nasal delivery in allergic rhinitis	69 70
Nanoparticles	Gellan–chitosan Gellan gum and pectin	Polyelectrolyte complexation Nebulization and ionotropic gelation method	Anti-fungal activity Colon specific delivery of resveratrol	71 72

8.7.1 Gellan Gum-based Beads

Nandi *et al.* described the development of calcium cross-linked hydrolyzed methacrylamide-*grafted*-gellan gum and tamarind seed gum (TSG) composite-based microbeads for prolonged release of diclofenac sodium.⁴⁶ Gellan gum was first copolymerized with methacrylamide employing a microwave-assisted free radical initiation method followed by partial hydrolysis with sodium hydroxide to obtain hydrolyzed polymethacrylamide-*grafted*-gellan (h-Pmaa-*g*-GG). This copolymer was mixed with tamarind seed gum and beads were prepared using an ionotropic gelation method (Figure 8.2). The experiment was designed by 3² full factorial design and the independent variables (polymer ratio and concentration of cross-linker, CaCl₂) were optimized by numerical optimization using Design-Expert software. The optimized batch exhibited a drug entrapment efficiency of 93.25%. The investigation reported a proportional decrease in drug release with decreasing ratio of polymers. An increase in calcium chloride concentration indicated increased release of diclofenac sodium. The drug release was reported to prolong over 10 h with a dissolution similarity factor (f_2) of 80.13 with a USP reference release profile. Kinetic modeling of the *in vitro* drug-release data demonstrated the drug-release mechanism based on case-I-Fickian diffusion.

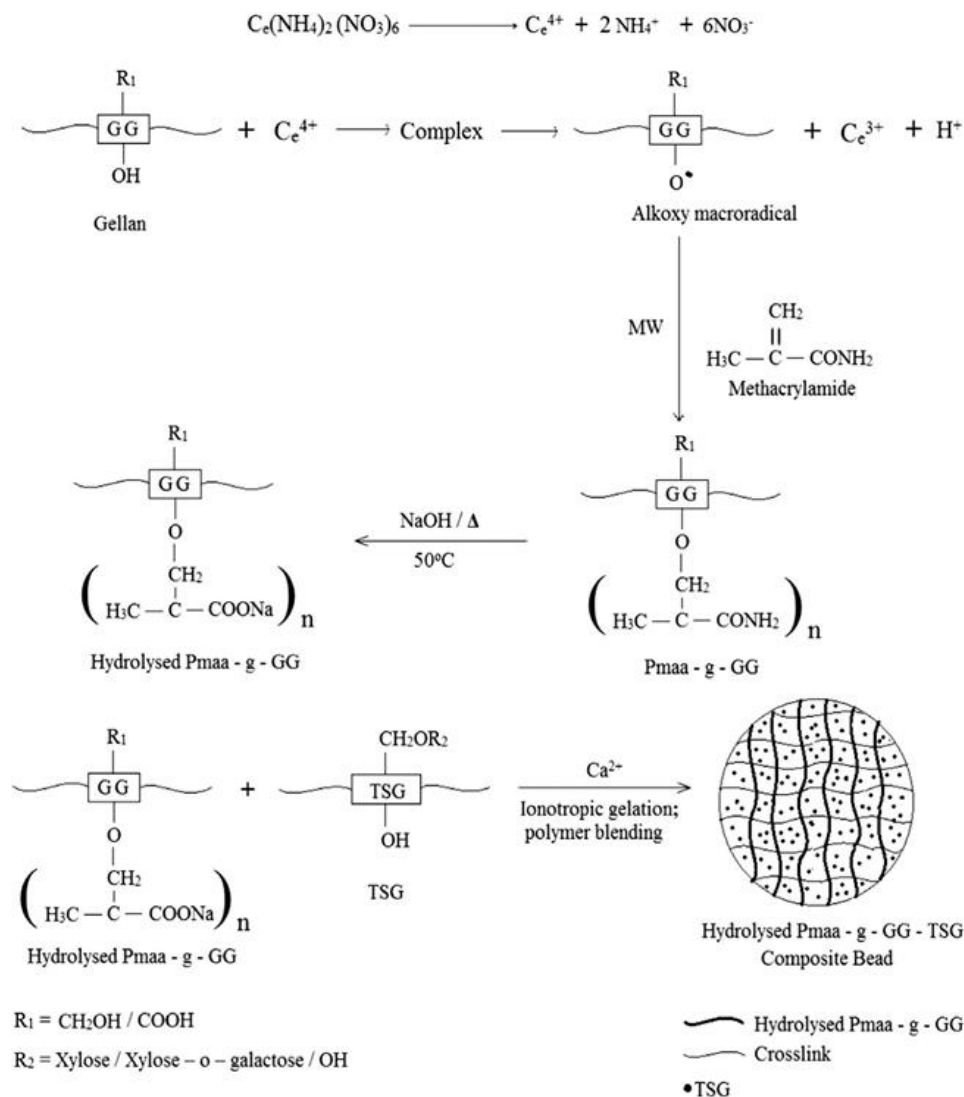


Figure 8.2 Schematic representation of the formation of hydrolyzed polymethacrylamide-*g*-gellan-tamarind seed gum beads.⁴⁶ Adapted from ref. 46 with permission from Elsevier, Copyright 2018.

Bera and coresearchers described other gellan-based beads, where a novel chemically modified polysaccharide (carboxymethyl fenugreek galactomannan, CFG) was incorporated in an ion-cross-linked gellan gum matrix. The major objective of their investigation was to fabricate a multiple-unit based controlled release oral delivery system of glimepiride.⁴⁷ CFG with the degree of carboxymethylation of 0.71 was synthesized and used in the beads. They prepared glimepiride-loaded beads through an ionic gelation method using bivalent (Ca²⁺ or Zn²⁺) or trivalent (Al³⁺) cross-linkers. 97% of maximum drug entrapment was reported exhibiting a sustained drug release profile following zero order kinetics with an anomalous diffusion mechanism. Polymer-blend ratios, cross-linker types, and incorporation of calcium silicate were found to influence the drug entrapment efficiency and sustained-release behaviors. *Ex vivo* study indicated the excellent mucoadhesive behavior of the beads. The study performed in diabetic rats exhibited significant hypoglycemic activity.

Another report demonstrated gastreretentive mucoadhesive ion-cross-linked beads of gellan gum for sustained-release of metformin hydrochloride.⁴⁸ In the study, starch extracted from jackfruit

(*Artocarpus heterophyllus* Lam., family: Moraceae) seeds was incorporated in the ion-cross-linked gellan matrix. The beads were prepared employing an ionotropic gelation method.

The polymer ratio and concentration of the cross-linker (CaCl_2) were optimized using 3^2 full factorial designs to enhance drug entrapment and restrict the release of drug after 10 h. The optimized beads showed excellent drug entrapment efficiency of $92.67 \pm 4.46\%$ and only 61.3% drug release at 10 hours indicating the sustained drug release ability of the beads with zero order kinetic and super case-II transport based drug-release mechanism. The optimized formulation demonstrated pH-responsive swelling. A mucoadhesive study was performed using intestinal mucosa of goats and revealed excellent mucoadhesive behavior. An *in vivo* study performed in diabetic rats exhibited a sustained hypoglycemic effect after peroral administration.

Development of gliclazide encapsulated modified release beads of ionically-cross-linked sodium alginate in combination with pectin or gellan was reported by Prajapati *et al.*⁴⁹ The study showed that an increase in the polymer concentration increases the swelling and drug encapsulation efficiency. The beads with sodium alginate and pectin in a 2 : 1 ratio and the beads composed of sodium alginate and gellan gum in the ratio of 6 : 0.75, showed initially high *in vitro* release of gliclazide followed by sustained-release through a diffusion mechanism.

8.7.2 Gellan Microparticles

Jana *et al.* described the preparation of gellan gum and alginate-based microspheres intended for sustained aceclofenac release. They prepared the microspheres through an unsaturated esterification process using maleic anhydride.⁵⁰ The microspheres showed a high degree of drug encapsulation efficiency of 98% with *in vitro* sustained drug-release over 6 h following the Korsmeyer–Peppas model. The average particle size of the microspheres was found within the range from 270 to 490 μm . Study findings indicated incorporation of maleic anhydride (3% w/v) has restricted the release of aceclofenac. The study report suggested an increased gellan gum concentration resulted in aceclofenac release following non-Fickian diffusion. Admirable anti-inflammatory efficacy was evidenced through an *in vivo* study performed on rabbits for a long duration.

Another report demonstrated the preparation of gellan microspheres using a water-in-oil emulsion method.³ The formulation was optimized through central composite design to achieve an average diameter around 300 μm . The mechanical strength of the microspheres was reinforced by cross-linking with different counter-ions. The optimized condition was obtained, which included 1.27% gellan gum with a stirring speed of 750 rpm. The nickel cross-linked gellan gum-based microspheres showed efficient encapsulation of different model proteins.

An anti-migraine drug almotriptan maleate is found to induce nausea and vomiting upon oral administration. Abbas *et al.* suggested intranasal delivery of almotriptan maleate through gellan microspheres to minimize these side effects.⁵¹ They described the preparation of gellan gum microspheres by a simultaneous water-in-oil emulsification and cross-linking method. The study was designed and the preparation conditions were optimized by 2^3 full factorial design. The drug–polymer ratio, percentage of cross-linker (CaCl_2), and time of cross-linking were selected as variable factors in the study, while particle-size and *in vitro* mucoadhesion were considered as response variables. The spherical shaped microspheres showed nearly 90% drug entrapment efficiency, excellent mucoadhesion and drug-release with a non-Fickian diffusion mechanism.

Other gellan microspheres were described by Boni *et al.*, where the microspheres were developed through an ionic gelation technique. Trivalent Al^{+++} ions were used as the cross-linker.⁵² The maximum drug entrapment efficiency of 87% was exhibited by the gellan microspheres and an ANOVA study demonstrated that increased polymeric concentration with a reduced concentration of cross-linker resulted in a positive impact on drug entrapment efficiency. The average size was within range from

700.17 to 938.32 μm . An increase in the particle size was accomplished by the increase in polymer concentration and crosslinker concentration. The microspheres showed excellent *ex vivo* mucoadhesiveness, pH-independent high swelling, pH-dependent drug release with the Weibull kinetic model, and a complex mechanism involving the swelling and erosion of the matrix.

Another mucoadhesive nasal formulation based on gellan microspheres has been reported to increase the residential time and drug absorption from the nasal mucosa, in which donepezil hydrochloride was used as model drug.⁵³ The microspheres were prepared using a conventional spray drying method with gellan gum. Polymer concentration and the liquid feed flow rate were optimized by 3^2 full factorial design in the preparation of the microspheres and their effect on drug entrapment, drug loading, and particle size was analyzed. The optimized microspheres showed excellent mucoadhesiveness, nontoxicity, and *ex vivo* permeation of the drug.

Methotrexate-loaded microparticles composed of gellan gum were described by Dhanka and co-researchers.⁵⁴ They prepared bare microparticles composed of native gellan by a W/O emulsification-solvent diffusion technique. The synthesized microparticles were reported to have a spherical shape. The microparticles showed a significant drug encapsulation efficiency of nearly 85% and thermogravimetric analysis showed slightly higher thermal stability of drug-loaded gellan microparticles compared to that composed of gellan gum alone. The *in vitro* drug-release study exhibited 84% methotrexate release for up to 24 h. A hemolysis study performed on human red blood cells showed less than 1.0% hemolysis. The cell viability test on L929 exhibited the biocompatible nature of methotrexate-loaded gellan microparticles.

8.7.3 Gellan Pellets

The use of gellan gum in pellet formulation as a binder was described by Barbosa and co-researchers.⁵⁵ The binding capacity of gellan gum was compared with polyvinylpyrrolidone in the study and theophylline was taken as the model drug. The study was designed by 3^2 factorial design with two factors and their three levels (binder and diluents). Pellets were prepared by the extrusion/spheronization technique. No significant effect of the binder on the pellet size and granulometric distribution was found in the study. More spherical pellets were observed in formulations with gellan gum, and the sustaining effect of gellan gum on the release profile was also observed, which justified the use of gellan gum in the formulation of sustained-release pellets as the binder as well as release-retardant.

8.7.4 Gellan-based *In Situ* Gel

In situ mucoadhesive gels based on ion-activated gellan for nasal delivery of salbutamol were described by Salunke *et al.*⁵⁶ The intention of nasal delivery was to shorten the onset of drug action. A mixture of two polymer gellan gum and hydroxypropylmethyl cellulose was used in the formulation. The gel formulated was capable of forming an *in situ* gel in the presence of physiological ions present in nasal mucosa. The physiological pH found in the nasal cavity was achieved in the formulation with maintenance of optimum viscosity and flow-ability for ease of nasal instillation. The study exhibited the major contribution of hydroxypropylmethyl cellulose in the mucoadhesiveness of the gel. The *in vitro* drug release study showed 97% drug-release in 11 hours. No histological damage on the nasal mucosa was observed in the *ex vivo* permeation study. They concluded that gellan gum-based *in situ* gels might be a potential approach for delivering salbutamol sulfate for intranasal administration.

An ion-activated ophthalmic preparation of ketotifen was developed with deacetylated gellan gum by Zhu *et al.*⁵⁷ The rheological characteristics, stability, *in vitro* gelation, *in vitro* drug-release, and pharmacodynamic outcome of the preparations were investigated in the study. An optimum viscosity was maintained in the formulation to ease the instillation into the eyes, but after instillation the sol

preparation was found to undergo gelation in conjunctival due to interaction between the ions present in lachrymal fluid and the sol preparation. The stability study exhibited no significant change in viscosity upon storage for 6 months. The *in vitro* study exhibited a sustained release of ketotifen from the formed gel matrix. Enhanced residence time in the eye was also found in the scintigraphic study. The *in situ* gel formulated with deacylated gellan exhibited a sustained and prolonged drug-action profile compared to the common ophthalmic drops.

Kotreka *et al.* reported the development of an ion-activated *in situ* ophthalmic gel loaded with estradiol to prevent cataracts.⁵⁸ The developed gellan gum-based ocular *in situ* gel was formulated using mannitol, polysorbate-80, disodium edetate dihydrate, and potassium sorbate. Interaction with a simulated tear solution resulted in a sol to gel transition which was evidenced by viscoelastic behavior. An *in vitro* study exhibited 80% estradiol release in 8 h with a non-Fickian diffusion mechanism. Retention of the physical and chemical stability was exhibited by the formulation.

A raft-forming gastroretentive *in situ* floating gel composed of gellan gum was described by Rao *et al.*⁵⁹ The study was performed for the formulation and optimization of itopride HCl loaded floating *in situ* gel for controlled release. Native gellan gum was used as an *in situ* gel forming polymer, calcium carbonate as the cross-linker, and hydroxypropylmethyl cellulose (HPMC K100M) as the sustained-release polymer. The formulation was optimized by 3² factorial design. The results revealed that gellan gum and HPMC K100M concentrations significantly influenced the viscosity and drug-release rate. The raft gel exhibited a controlled drug-release profile with a Korsmeyer–Peppas kinetic model. *In vivo* study revealed relatively higher $T_{\max}$ from the *in situ* gel compared to the plain drug suggesting slower absorption and 90% higher AUC_{0–12h} than the plain drug.

Jelkmann and co-researchers recently reported the development of a novel gellan gum derivative-based nasal *in situ* gel with a mucoadhesive property.⁶⁰ In their study, amino groups were introduced to the gellan gum polymeric backbone. Mucoadhesion was evaluated by studying the rheological synergism with a rotating cylinder and tensile strength. The erythrocyte-/cytotoxicity evaluation test revealed compatibility of the gellan derivative with nasal epithelial cells. The study revealed 32-fold viscosity enhancement of a modified gellan gum–mucin mixture and 14-fold increase in adhesion time with the mucus membrane, which indicated the increase in mucoadhesion compared to native gellan. Tensile study exhibited a 9-times increase in adhesive force and 3.75-times elevation in detachment force. The overall results substantiated the application of aminated gellan gum as a promising mucoadhesive polymer suitable for nasal drug delivery.

8.7.5 Tablets

Metronidazole loaded tablets composed of gellan gum were prepared and evaluated for swelling and disintegrating properties of gellan gum.⁶¹ The study was conducted using both simulated gastric and intestinal fluid. The mechanical properties such as hardness, friability, as well as the disintegration and drug-release profile of the tablets were also evaluated and compared with those of two standard disintegrating agents such as maize starch and sodium starch glycolate. The results exhibited a rapid rate with a higher degree of swelling in the presence of basic medium than acidic medium. The study also showed that extra-granular incorporation of gellan gum at the concentration of 0.2% w/w was most effective as a tablet disintegrating agent for metronidazole tablets.

The binding capacity of gellan gum in tablet formulation was studied in paracetamol or metronidazole containing tablets.⁶² The efficiency of gellan gum as a binder was investigated with respect to gelatin and acacia. In the study, tablets were prepared using a conventional wet granulation technique. The results of the study exhibited that gellan gum had lower cohesiveness and adhesiveness than that of gum acacia and gelatin. The *in vitro* drug release study revealed faster release of metronidazole from tablets composed of gellan gum than that of gelatin or acacia but the release of

paracetamol from the tablets composed of gellan was found to be slower than that from the tablets composed of gelatin or gum acacia. The overall results of the investigation designated that in spite of the reduced hardness of gellan gum-based tablets, they could be used in conventional tablet formulation with medium hardness, low friability, and sufficient disintegration and drug release properties.

Priyadarshini *et al.* formulated metformin hydrochloride gastroretentive sustained release tablets based on polymethacrylamide-grafted-gellan and a tamarind seed gum matrix.⁶⁴ By varying the polymeric composition, tablets were prepared following a wet granulation technique. Sodium bicarbonate was employed as a buoyant material. Several evaluation tests including minimum buoyancy-lag-time, maximum mucoadhesive strength, and USP reference drug release profile were performed. The optimized tablet formulation showed a very short floating-lag-time of 2.76 min, excellent buoyancy over a 10 h period, significant mucoadhesion, extended release of metformin hydrochloride over 10 h with an anomalous non-Fickian transport-based drug release mechanism.

Cilnidipine, an anti-hypertensive fourth-generation calcium channel blocker (both L- and N-type) has been used in the management of hypertension as a safe and effective drug without reflux tachycardia compared to other dihydropyridine analog calcium channel blockers. However, poor aqueous solubility and hepatic first-pass metabolism are the causes of poor bioavailability through an oral route. A gastroretentive tablet formulation of this newer anti-hypertensive drug based on gellan gum has been recently described with the objective to enhance the oral bioavailability.⁶³ A combination of floatability through intra-matrix gas generation and mucoadhesiveness has been adopted in the gastroretention strategy of the study. A direct compression technique was employed to prepare tablets using sodium bicarbonate as the gas generating agent. ANOVA study showed that gellan gum has a significant influence on mucoadhesive strength and drug release. An *in vitro* dissolution study showed extended release of cilnidipine over 12 h with anomalous-diffusion based on combined diffusion and erosion. An *in vivo* study in human volunteers with radio-opaque tablets exhibited gastro-retention in the stomach for a period over 6 h with required buoyancy and mucoadhesion. Pharmacokinetic analysis conducted in human volunteers demonstrated enhanced relative bioavailability from the proposed gastroretentive tablets composed of gellan gum compared to reference tablets.

8.7.6 Hydrogels

Mahmood *et al.* reported the development of hydrogel films of gellan gum using an ionic gelation method for co-incorporation of ofloxacin in combination with volatile oil as a wound dressing.⁶⁵ The flexible and transparent films exhibited antioxidant as well as antibacterial efficacy against common Gram positive and Gram negative bacterial inhabitants of wounds. A dissolution study indicated a rapid release of drug initially followed by a controlled release of the remaining payload over a period of 48 hours from the films. No negative influence of the oil present in the film on the release of ofloxacin was found in the study. The optimized film formulation with ofloxacin and tea tree oil (25% w/w) exhibited considerable contraction of wounds (98%) in rats after 240 h of administration. Histological study also exhibited an image of a completely healed epidermis.

Another gellan gum-based sustained-release hydrogel was demonstrated by Zhang *et al.*⁶⁶ The proposed hydrogel was intended for antibacterial action in wound healing. In this study, a novel composite hydrogel for wound dressing aimed to prevent wound infection, which was formulated with gellan gum microspheres interspersed into a doubly-crosslinked hydrogel. The dual cross-linked hydrogel formulation was prepared by Schiff-base cross-linking of oxidized gellan which was previously cross-linked by Ca^{2+} ions and modified chitosan. Hydrogel-based formulations were optimized by varying the oxidized gellan, carboxymethyl chitosan, and Ca^{2+} ion ratio. The best hydrogel formulation was found to contain carboxymethyl chitosan and oxidized gellan with a ratio of 16 : 7. The optimized formulation was found to exhibit a gelation time of 139 s, swelling index of 30 after swelling equilibrium, rate of degradation of 100.5% on the seventh day and a compressive

modulus of 8.8 kPa, and a sustained release profile of tetracycline hydrochloride and silver sulfadiazine. *In vitro* antibacterial study showed excellent antibacterial activity of the composite hydrogel. The overall results of the study substantiated the application of the gellan-based hydrogel as an effective injectable scaffold in drug delivery and wound healing.

A gellan–chitosan based hydrogel was formulated for delivering apigenin.⁶⁷ Polyethylene glycol was used in the formulation as a cross-linker. Apigenin was extracted from the leaves of *M. alba* which was screened using an *in vivo* wound on a rat model.

Apigenin was isolated from ethanolic extract of *M. alba* leaves and screened by *in vivo* wound models in rats. The performance of the hydrogel was tested for wound healing activity with parameters such as antioxidant efficacy, wound contraction, weights of dried granuloma, and collagen content. The drug encapsulation efficiency was reported as 87%. An *in vitro* drug-release study showed controlled release of apigenin with 96% release in 24 hours. Study findings suggested increased levels of catalase and superoxide dismutase for the apigenin treated group. Better wound healing efficacy was observed in diabetic and normal wounds.

Prevention of infection spreading in the skeletal system in osteomyelitis requires prolonged delivery of antibiotics in high doses. However, the administration of antibiotics in high doses through systemic routes is inconvenient and produces several side effects. A novel injectable vancomycin-loaded nanoparticulate delivery system was described by Posadowska *et al.* for local vancomycin delivery.⁶⁸ Vancomycin was first incorporated in poly(l-lactide-co-glycolide) (PLG) NPs employing a double-emulsification method. In order to make the nanoparticles injectable, the nanoparticles were dispersed in an aqueous solution of gellan gum and cross-linked with calcium ions. In this step, an amount of vancomycin was incorporated in the system extraparticularly. The resulting system exhibited suitable rheological property for injectability, initial rapid release, and then prolonged vancomycin release. The injectable system was also found to be cytocompatible with osteoblast-like MG-63 cells and active against reference strains of *Staphylococcus* spp. as well as those isolated from osteomyelitic joints.

8.7.7 Micelles

Gellan gum-based micelles were described by Kundu *et al.*, where gellan gum was conjugated with alkyl groups ($C = 16$) in order to introduce an amphiphilic character to native gellan gum and subsequently fabricated in drug-loaded micelles.⁶⁹ The main objective of their study was to solubilize a very poorly water-soluble drug, simvastatin. The alkylated gellan gum was found to form self-assembled spherical micelles with a mean diameter of 832 nm when added in water. The study revealed a significant rise in the aqueous solubility of simvastatin due to micellar encapsulation of the drug molecules. Furthermore, the micelle-entrapped simvastatin and its pure form were separately encapsulated in beads of aluminum cross-linked gellan hydrogels. A significantly higher drug encapsulation efficiency of around 94% was exhibited by the beads. The beads also exhibited faster dissolution of simvastatin in phosphate buffer of pH 6.8 than that in HCl acid of pH 1.2. The drug dissolution from the beads were reported to be affected by medium pH and the swelling character of the gellan beads. The kinetic modeling of the drug release data suggested an anomalous diffusion-based mechanism of drug release. Finally, an *in vivo* study in a rabbit model exhibited 83.45% reduction in blood LDL-cholesterol level following 18 h of oral administration of the micelle-encapsulated gellan beads, which substantiated the application of micellar encapsulation of poorly water-soluble drugs in enhancement of the dissolution profile as well as pharmacodynamics.

Maiti and co-researchers described another gellan copolymer-based micellar encapsulation of an anti-rhinitis drug budesonide.⁷⁰ In the study, gellan gum was grafted with a long alkyl chain (C_{18}) by etherification reaction. The alkylation of the polysaccharide was found to impart an amphiphilic character to gellan gum and the dispersion of this modified gellan gum in water resulted in spontaneous formation of self-assembled spherical micelles in nanoscale. A maximum drug loading greater than 95%

was exhibited in the study. The size of the nanomicelles was found between 371–750 nm. The reported zeta potential of –48.3 to –67.2 mV indicated stability of the nanomicelles in aqueous dispersion due to significant repulsion between the micelles. The *in vitro* drug release study exhibited a prolonged drug dissolution profile with first-order kinetics in 5.5 pH simulated nasal solution. The maximum dissolution rate was found to be around 40% at the lowest polymer : drug ratio in a period of 6 h. The fitting of the dissolution data to the Korsmeyer–Peppas model indicated drug release only by simple diffusion. This type of chemical modification of natural polysaccharides and subsequent micellar encapsulation of drugs like budesonide might be beneficial for nasal delivery of anti-rhinitis drugs in better management of allergic rhinitis.

8.7.8 Gellan Nanoparticles

Kumar *et al.* demonstrated the preparation of ketoconazole loaded nanoparticles (NPs) composed of gellan gum and chitosan for antifungal efficacy.⁷¹ The drug-loaded NPs were prepared using a polyelectrolyte complex formation method employing gellan gum in combination with chitosan. Gellan gum was found to influence the particle size of the nanoparticles more prominently than chitosan. Finally the study exhibited significantly higher antifungal activity of ketoconazole loaded gellan–chitosan NPs against *A. niger* in comparison to placebo NPs and pure ketoconazole separately.

Gellan gum–pectin-based colon targeting NPs of resveratrol were described by Prezotti *et al.*⁷² The effects of the polymers on the release behavior and gastro-intestinal permeability were investigated. The polysaccharide-based NPs were synthesized by nebulization and ionic gelation method. The maximum resveratrol encapsulation was found to be greater than 80%. *In vitro* drug release study exhibited minimal resveratrol release (3%) in acidic pH in 2 h and 85% in a sustained manner in pH 6.8. The permeability of drug-loaded nanoparticles in the Caco-2 model was found to be 0.15%, but 5.5% in the presence of mucus in the triple co-culture model. The everted sac investigation ratified the lower permeability of the NPs with or without the presence of mucus indicated their higher affinity towards the intestinal membrane. The study indicated the safety and controlled colon specific delivery of resveratrol from polysaccharide-nanoparticles.

8.7.9 Gellan as an Emulsion Stabilizer

A study described the application of high acyl gellan as an emulsion stabilizer in an oil-in-water emulsion formulation of edible sunflower oil.⁷⁴ The study reveals that high acyl gellan can stabilize the emulsion, whereas, low acyl gellan cannot. The physical properties of the emulsion were found to be dependent on the process of mixing of oil with a dispersion of gellan gum. Homogenization under increased pressure favored the stabilization, whereas, application of high energy ultrasound resulted in destabilization. A zeta potential of –50 to –59 mV was reported for the emulsion prepared through a homogenization technique under high pressure. High acyl gellan also increased the viscosity of the continuous phase, which may contribute to the stability along with a negative zeta potential. The increased viscosity of the dispersing phase might attribute to the presence of aggregates or microgels. It was observed that disentanglement of these aggregates by ultrasound reduced the viscosity of the continuous phase, which affected the stability of the emulsions.

8.7.10 Gellan-based Nanofiber

Rostami *et al.* reported resveratrol-loaded gellan gum and chitosan-based nanofibers.⁷³ The novel nanofibers were prepared using an electro-spinning technique with chitosan in trifluoroacetic acid and gellan gum. The diameters of gellan gum–chitosan nanofibers were found to be 166 ± 37 and 291 ± 41 nm from the ratios of chitosan : gellan of 95 : 5 and 90 : 10, respectively. The maximum drug encapsulation efficiency was reported as 86%. The *in vitro* dissolution study exhibited 43 to 51% drug

release in intestinal pH. The study also exhibited significantly higher antioxidant activity of resveratrol-loaded nanofibers compared to free drug. Furthermore, the MTT assay revealed equivalent cytotoxicity of the drug-loaded nanofibers against HT29 cancer cells compared to free resveratrol.

8.8 Conclusion

The bacterial exopolysaccharide, gellan gum has been extensively studied for its various applications in drug delivery, food, and biomedical fields including tissue engineering and cell entrapment due to its unique physicochemical and thermosensitive properties along with its biocompatible and biodegradable nature. This natural polysaccharide has been explored in a wide range of drug delivery systems including tablets, capsules, emulsions, suspensions, beads, microparticles, nanoparticles, and hydrogel films as wound dressings, *etc.* Various chemical derivatives of gellan have also been explored in order to customize the drug delivery. Gellan gum is also being explored in an emerging field of cell encapsulation for different biomedical applications.

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Chapter 9

Locust Bean Gum – A Potential Drug Delivery Carrier

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9.1 Introduction

In current years, plant-derived polysaccharides from various natural sources have attracted much attention, mainly towards biochemistry and pharmacology applications. Galactomannans are polysaccharides with a firm hydrophilic backbone (polymannose or mannan) and grafted galactose units with many beneficial properties. In the plant world, concerning their distribution, galactomannans are biopolymers that comprise the second largest storage polysaccharides. They are natural polysaccharides with great attention in the pharma sector due to their wide variety of applications and are found in the endosperm of seeds in plants.^{1–4} In general, seed-derived galactomannans are referred to as seed gums that are biodegradable and biocompatible biopolymers.⁵ The common leguminous and convolvulus plants have seed gums, but these gums' structure and properties vary from

species to species. They are heterogeneous polysaccharides consisting of an α -d-mannopyranosyl backbone(1 $\rightarrow$ 4)-linked, substituted by β -d-galactopyranose at O-6 single units. Based on the plant source, the mannose/galactose (M/G) ratio differs from 10 to 1. With the collective galactose content, the water solubility of galactomannans rises, particularly with a reduced ratio of M/G. The side chain residues of α -d-galactopyranose determine its solubility. For its solubility in water, a 10% galactose substitution level is essential, and due to the non-substituted regions of the polymer,⁶⁻⁸ agalactose content <10% precipitates at room temperature. At reduced temperatures, for the entire gum the solubility is narrowed to the polymer chains where the galactose amount is greater than the average value, and fractions of various M/G ratios can be attained at increasingly greater temperatures.

For human consumption, galactomannans are available in diverse forms. Due to their various physicochemical properties, they can be functional materials with numerous applications. They are active viscosifiers and thickeners in aqueous systems, and furthermore, they are very good stiffeners and emulsion stabilizers. Their biocompatible, biodegradable, and non-toxic nature increases their use in the pharmaceutical, biomedical, textile, and cosmetics industries. As crude flour, pharmaceutical technology and cosmetics mainly used galactomannan, which is not as pure. 85% pure galactomannan is seen in commercially sold flours on a mass basis and had vital involvement in the hydrocolloid usage in many products.⁹⁻¹¹ In recent times, there has been a global trend to discover new plant-derived biomaterials and explore them to develop products as plant-based products are considered 'generally recognized as safe' (GRAS).¹² Moreover, their significance has dramatically improved due to their eco-friendly processing without violating strict government regulations on environmental pollution by the pharmaceutical industry.^{13,14}

Like dietary fibers, galactomannans are also consumed increasingly with atoxic bioactivities to lower calorie intake, reduce weight, control blood glucose, cholesterol, and insulin levels, and decrease the possibility of heart diseases and colon cancer. They are used as texture modifiers and stabilizers in specialty foods.¹⁵ For gel formation with novel properties, galactomannans have occasionally been used with other polysaccharides.

Recent international trends claim the development of substitute sources of gums from seeds and essential exploration of other renewable sources, for example, pharmaceutical materials. In Latin America, despite the abundant biodiversity of the native flora and the appropriate climate, the known sources of galactomannans are negligible for their production. In the food and pharmaceutical industries, the three commercially essential galactomannans are guar (*Cyamopsis tetragonolobus*), tara (*Caesalpinia spinosa*), and locust bean gum (*Ceratonia siliqua*) with M/G ratios of 1.5, 3, and 3.5 respectively. Other commercial galactomannans, such as gums of *Cassia tora* (M/G 8.3), *Trigonella foenumgraecum* (M/G 1.0), and *Prosopis juliflora* (M/G 1.2), are also manufactured.

Over the last decade, there have been studies reported from various plant sources based on the composition of galactomannan and their physiochemical properties. Even so, only cassia gum, guar gum, locust bean gum (LBG), and tara gum are produced on a large scale. Srivastava and Kapoor reported by chemical screening over 300 water-soluble gums, which are Indian species in their summary of galactomannans.¹⁶ The already isolated and structurally characterized immense numbers of galactomannans that offer pharmaceutical and other industrial applications provide an exciting challenge.

Plant resources are renewable and supply a constant amount of raw materials if cultivated or harvested sustainably, which is the utmost characteristic of plant resources. Still, plant-based materials also constitute possible challenges such as their manufacture in minor amounts, and generally complex structures, which may differ based on plant location. Other reasons like the season for plant material collection might influence its distinctive features resulting in moderate and costly separation and purification processes.¹⁷ From this respect, reports show that depending on the kind of cultivar, growth environments, or other unknown biological characteristics, the chemical composition and molecular weight of LBG differ systematically.^{11,17} This possibly justifies why LBG is considered polydisperse. Polydispersion can mainly provide three types of variation in the structure of the polymer; substitution of galactose, side group patterning of galactose, and polymerization degree.¹¹ The most important fact is that diverse degrees of mannose chain substitution will influence the solubility

of the LBG out of other suppliers.^{18,19} This may be one of the main disadvantages that hinder a characteristic application of LBG in the biopharmaceutical area, as one of the significant characteristics to be controlled is its solubility, and under definite conditions, it should be essentially stable.

9.2 Locust Bean Gum

9.2.1 Manufacture

From the endosperm of the locust plant, LBG is retrieved. Among the Leguminosae family, it belongs to the Caesalpinioideae subfamily.²⁰ This tree belongs to semiarid environments and is plentiful in the Mediterranean area, different areas of Spain, Italy, Cyprus, and other Mediterranean countries, North Africa, South America, and Asia.²¹ Furthermore, familiar locust bean manufacturing nations are India, Algeria, Morocco, Greece, Pakistan, Israel, and Turkey. It is an enduring evergreen tree, and in 10–15 years it reaches approximately 10 m in height. At the age of 15 years it starts to bear pods. The pods of the locust bean tree produce large brown sickle-shaped fruits known as carob pods. For high yields, hot and dry climatic environments are essential. The pods attain their maximum size in July but are ready in October. Per annum, a half-ton of pods can be harvested from large trees. The average length of carob pods is 10–20 cm with a width of 2–4 cm and has 10–15 carob seeds or kernels with an oval shape. From two lakhs hectare, approximately 315 000 tons of carob seeds are the estimated worldly production per year, and the chief producers comprise Morocco (38%), Spain (28%), Italy (8%), Portugal (8%), Greece (6%), Turkey (6%), and Cyprus (2%).²² The endosperm of the locust tree from where the polysaccharide is acquired is called the LBG, locust seed gum, locust flour, or *Ceratonia*.²³ Recently, owing to its many industrial and pharmaceutical uses, the importance of LBG has been increasing widely. LBG is used in food as a stabilizing food additive and thickening agent, and in pharma industries, it is used in tablets as controlled release excipients.^{18,24} The main features such as biodegradability, low toxicity, and small cost contribute to the growing use of LBG in various fields. The

estimated annual production of LBG (LBG-E 410) worldwide alone is 15 000 tons, and the present prices are from 12 to 22 euros per kg or more depending on the grade and supplier of LBG.²⁴

9.2.2 Processing

Locust bean seeds are embedded in a yellowish pulpy material, and the pods are ground for seed detachment from the pulp. The husk, endosperm, and germ are the three portions of the seeds. The processing of locust bean seeds includes different steps like removing the husk from the grains, splitting the seeds, milling, sifting, clarification, and finally drying the seeds. The hard and rigid seed coat of the locust seeds makes the processing very tough. Using dilute sulfuric acid or a thermo-mechanical treatment known as acid peeling and thermal peeling, the dehusking processing of locust bean kernels is completed. In the acid peeling method, seeds are treated with sulfuric acid at high temperatures to carbonize the seed coat. Then the residual part of the seed coat is detached from the endosperm, and the peeled kernels are dehydrated, broken, and macerated. From the undamaged endosperm halves, the germ portions are selected, and they are known as splits. The LBG made from the splits is whitish and has greater viscosity.²²

In the thermo-mechanical peeling process, rotating furnaces are used to roast locust kernels wherefrom the innermost part of locust kernels, the coat of seeds, departs. After mechanical processing the splits are acquired. To make a fine powder of LBG, the splits are milled and sieved. Owing to the heating or roasting operations, the powder of LBG from this procedure is darker in color. This process requires sulfuric acid and has no effluent. By dissolving it in water and by heating, clearing up of native LBG can be done. to obtain a clear solution, it is exposed to filtration to eliminate insoluble materials. In order to obtain a fine powder of purified LBG, it is retrieved by precipitation using solvents like isopropanol/ethanol, by different processes like filtering of LBG, drying, and grinding or milling of LBG (Figure 9.1).

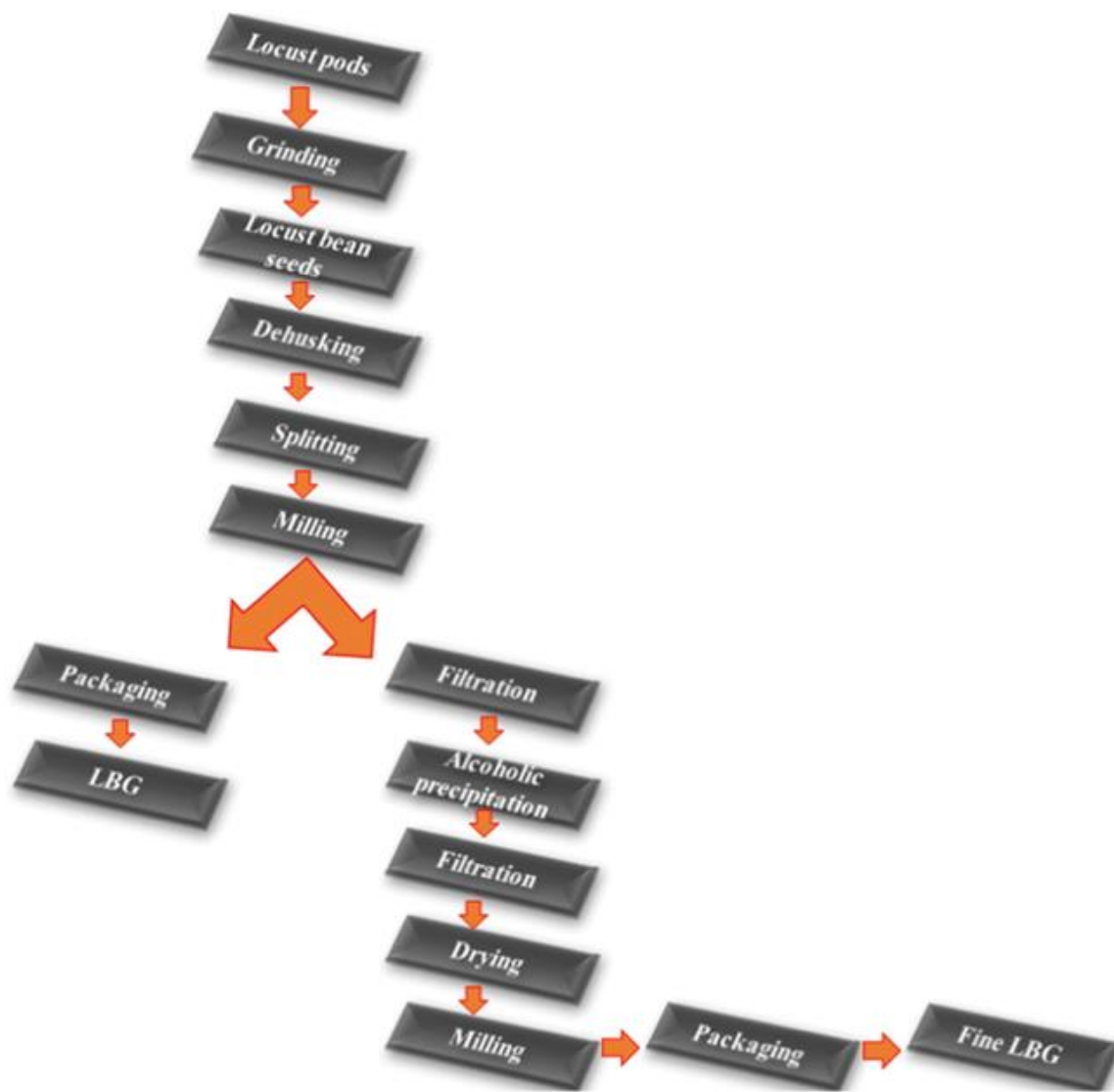


Figure 9.1 Flow diagram of the processing of LBG.

The main content of locust seeds is galactomannan (nearly 80%), and the remaining content is made up of proteins and impurities.^{25,26} Ash and acid-insoluble matter are the chief impurities reported,²⁵ and even when heating to 70 °C, the impurities generally persist as they are insoluble.²⁷ To eliminate the protein content and impurities, the crude galactomannan underwent various procedures, chiefly enzymatic hydrolysis or hydrolysis in alkaline solutions, precipitation with solvents like ethanol or isopropanol, and purification using methanol or copper/barium complexes.^{25,26,28} Isopropanol precipitation is used for the removal of proteins. Overall, the

purification stages have been confirmed with greater M/G ratios and decreased protein and impurity levels.²⁵

9.2.3 Composition

Depending on the weight of the seed, the kernel of the locust bean is made of three main parts; an outside husk (30–33%), germ (23–25%), and endosperm (42–46%). Galactomannan is the chief part of the endosperm in a locust bean, which includes approximately 80% of the weight and the remaining weight is from the proteins and impurities. The chief protein part of LBG comprises albumin and globulin (32%), and glutelin is the other 68%.²⁹ The marketable LBG composition consists of galactomannan (80–85%), moisture (10–12%), protein (5–6%), fat (0.5–0.9%), fiber (0.8–1%), and ash (0.5–1%).^{30,31} Husks and germ are the potential impurities in commercial LBG powder revealed by acid-insoluble matter and protein. Some ethanol/isopropanol from the washing/extraction processes also act as impurities in LBG fine particles.³⁰

9.2.4 Chemical Structure

LBG is an odorless white powder acquired after milling the endosperm of the fruit pod. LBG is a galactomannan made of two sugar units, *i.e.*, galactose and mannose. Chemically they consist of (1 → 4)-linked β -D-mannose linear chains with α -galactopyranose residues (1 → 6)-linked side chains, thus being neutral polymers.^{11,17,31,32} For LBG, the mannose/galactose (M/G) ratio is $\approx 4 : 1$, and it differs based on the galactose distribution over the mannose backbone. On the linear backbone, galactose attaches to the mannose chain randomly.³³ The M/G ratio is an essential factor in galactomannan solubility; a higher galactose content increases the water solubility.^{34,35} Based on the seed source, growth environments, and manufacturing procedures, the molecular weight usually ranges from 0.3 to 2.0 million. Molecular weight valuation methods like gel permeation chromatography and size exclusion chromatography determine the molecular weight, and the M/G ratio is mainly resolved using gas chromatography. There are reports that the molecular weight is approximately 310 000 for locust galactomannan and is polydispersed.^{36,37}

As it is non-ionic, LBG solutions are not affected by pH, salts, and heat treatment.

9.3 Properties of LBG

9.3.1 Solubility

Galactomannans are hydrophilic and several circumstances decrease their solubility. The functional characteristics of locust galactomannan rely on its nature in an aqueous medium as it is not entirely cold-water-soluble. In water (hot or cold), it develops a sol with a pH (5.4–7.0), which can be changed to a gel by adding minor quantities of sodium borate.³⁸ For solubilization in water, heating is essential. Heating beyond 80 °C may cause oxidative–reductive depolymerization of a chain of galactomannan. This process leads to a decrease in the viscosity of the solution. The LBG solubility does not go beyond 90%, which depends on factors such as particle size, granulation, and impurities.^{24,31,39}

9.3.2 Viscosity

The essential characteristic feature of LBG is the formation of viscous solutions when it is hydrated in hot water. LBG can develop viscous solutions that are almost unchanged by pH, salts, or temperature.³² The viscosity of LBG depends on several aspects like the size of the particle, concentration of the polymer, solubilization property, molecular weight of the polymer, and shear rate. Among other galactomannans, the intrinsic viscosity of the LBG and molecular weight are lower. The viscosity of solutions of LBG is dependent on its concentration and usually demonstrates an increase with gum concentration.

9.3.3 Molecular Weight

The reported molecular weight of LBG is approximately 50 000–3 000 000 g mol⁻¹ (Commission Regulation (EU) No. 231/2012).⁴⁰ Hydrolysis of high molecular weight galactomannans is achieved using two main methods: acid hydrolysis and enzymatic hydrolysis. The details of acid and enzymatic hydrolysis are described in the biodegradation of the LBG section.

9.3.4 Hydration Rate

Depending on the grades, the hydration rate of LBG differs. The grading of LBG is based on the size of the particle, the content of the gum, and solubility. When the gum powder particle size is thin, it hydrates effortlessly and much faster than a comparatively coarser particle size. For quick hydration commercially, fine-sized particles of LBG are available.

9.3.5 Water Adsorption Isotherm

The correlation between equilibrium moisture content versus water activity is the water adsorption isotherm. The water adsorption isotherm of LBG reported by Torres *et al.* (2012) showed an increase in moisture content at 20–65 °C. The isosteric sorption heat of LBG is reduced by increasing equilibrium moisture content. LBG demonstrates a great sorption heat of 27 kJ mol⁻¹ which shows the smaller amount of hygroscopicity of the gum.^{41,42}

9.3.6 Synergistic Gel Formation

As numerous products comprise the formulation of mixed polysaccharide systems, the synergistic properties among polysaccharides arouse great interest. Modulating the rheology of product synergies offers power that is highly significant in pharmaceutical preparations.⁴³ Two polymers (with gelling capacity or not) are combined in the gel formation process, and a synergic interaction occurs. This gelation process happens when a more specific interaction between the polymeric chains of materials begins, leading to an enhanced water absorption capacity related to the sum of the absorption of each material individually. Furthermore, this can occur by adding a minor amount of non-gelling polymer to a polymer with a gelling property that encourages the gel strengthening or, many polymers that alone are non-gelling but upon mixing can form gels. This nonadditive nature is named synergism. Various mechanisms influence the formation of synergistic gels.⁴⁴ When a covalent bond is formed among the polymer chains, a chemical gel network is formed.

Moreover, mixed junction zones between different polymers are included in physical gel formation or electrostatic interactions among diverse

polymeric chains. LBG shows synergistic interactions among other polysaccharides, chiefly due to several hydroxyl groups in their structure.⁴⁵ This property raises the polymer flexibility and allows the formation of a gelling network with pharmaceutical importance. The best synergies of LBG are well-known for their interaction with xanthan and carrageenan gum.^{46–49}

LBG does not form a gel; however, a weak gel can be found upon freeze–thaw treatment. In the presence of sucrose, LBG forms a gel. There is an early upsurge in the strength of the gel in the presence of sugar and a following reduction with rising sugar concentration. The early gel strength augmentation is possibly due to the decrease in the content of water and the augmented sugar amount. The successive reduction in gel strength leads to the restricted association between polymer–polymer, causing sugar molecules to bind to the polymer chains, leading to decreased gel strength. The highest gel strength of LBG solutions can be reached by 45% fructose, 50% sucrose or sorbitol, and 55% glucose by weight.⁵⁰ A very beneficial synergistic increase in gel strength shown by LBG upon interaction with gums like xanthan gum carrageenan *etc.* was reported. Rocks (1971) reported the synergistic interaction among polysaccharides for the first time, perceiving a thermally reversible gel formation.⁵¹ The reports revealed the reaction occurs as a lock and key model among the side chains of xanthan and backbones of LBG, such that one xanthan chain might assist with one or more LBG molecules.^{52,53} The LBG/xanthan synergic reaction is affected by the molecular weight of the polymers and the degree of substitution of LBG. A smaller amount of galactose substitution is very adequate for the development of a gel.

k-Carrageenan gelation leads up to the coil–helix transition, and subsequent accumulation and development of the network. This type of coil–helix transition is guided by various factors like the concentration of salt, electrolytes, polymer length, and temperature.^{54,55} The measurements in viscoelasticity and tests for compression show that to the network of *k*-Carrageenan, LBG attaches non-specifically. Besides this, there are reports that gels of *k*-carrageenan are syneresis susceptible.⁵⁶

9.4 Biological Activity of LBG

LBG belongs to the class of viscous soluble fibers and has various health benefits. LBG can modify the amount of carbohydrate degradation on digestion, thus regulating the blood sugar level and insulin level. In 1983, researchers reported that LBG has a hypolipidemic effect by reducing low-density lipoprotein levels due to its insoluble fiber content. Recent reports on gums as dietary fiber showed a beneficial role in cardiovascular diseases.^{57,58} Moreover, due to excellent gelling capacity and the gastrointestinal (GI) tract not adjusting to the formed gel, ingestion leads to a sense of satiety and reduced absorption of nutrients, making LBG acceptable for dietary products.⁴⁹ LBG-based capsule formulation is available on the market (Carob gum – Arkopharma Arkocápsulas^â) for suppressing appetite.

9.5 Biocompatible and Biodegradable Nature of LBG

Polymer–drug delivery systems (DDS) are used to make biocompatible and biodegradable DDS. In polymer metabolism, various chemical, physical, and biological processes are involved and result in polymer chain break up, leading to changes in solubility and molecular weight.

The biodegradation of LBG is chiefly by enzymatic activity (Figure 9.2). β -Mannanase in the human colonic region; the effective degradation of LBG, possibly by oral administration, is the basis of the upsurge in some approaches of colonic drug delivery (CDD). However, the change of polysaccharides into oligosaccharides comprises the complete breaking of its units. The enzymatic degradation of LBG happens by β -mannanase enzymes on α -mannose chains (β -(1,4)units), changing these components into mannobiose, mannotriose, and galactomannobiose.^{59,60} On mannose and galactose chains, β -mannosidase and α -galactosidase enzymes cause the complete degradation of LBG, forming α -mannose and α -galactose. Various studies reported on LBG *in vivo* degradation by colonic bacteria,⁶¹ an outcome explicitly credited to *bacteroides* and *ruminococcin*.⁶²

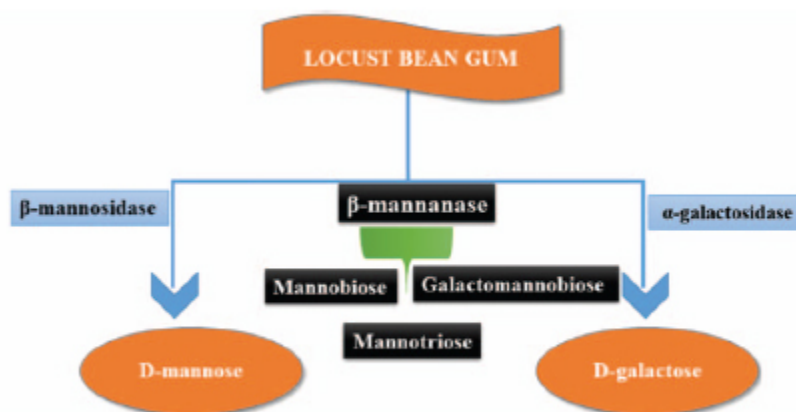


Figure 9.2 Biodegradation of locust bean gum with enzymes.

Depolymerization of LBG is tough due to the galactose residues' steric effect over β -mannanase. The depolymerization usually begins with the enzyme α -galactosidase by eliminating some residues of galactose and uncovering residues of the mannose for the β -mannanase action.³³ α -Galactosidase from plant, bacterial, and fungal origin is mainly used in industrial depolymerization.⁶³ α -Galactosidase fungal origin is more suitable for applications mainly because of the acidic pH and broad range of stability.

9.6 Drug Delivery Applications

Natural polymers have attracted tremendous attention and have been widely considered as vehicles for drug encapsulation and delivery. The therapeutic efficiency of a drug delivery system is related to several factors, mainly depending on pharmacokinetics, distribution, cell uptake, metabolism, excretion and clearance, and toxicity.⁶⁴ In the area of drug delivery (DD), in recent decades studies have been devoted to the progress of appropriate delivery systems for boosting the therapeutic efficacy that avoids or minimizes the side effects of the drug. Natural polymer-based DD materials show excellent biocompatibility, easy availability, and can be easily modified. Furthermore, native natural polymers have various reactive groups. Additional functional groups could also be introduced to the natural

polymers, thus providing the newly attained polymer with significant functions or can regulate their physical and chemical properties.^{65,66} In DD applications polysaccharides and proteins are widely used. Besides this, the backbones of the polysaccharides have ample functional groups for modification that includes hydroxyl groups allowing them easy availability for more modifications.^{67–69}

In DD, the application of LBG is variable and used for producing various DD agents such as tablets, gel, films, microspheres, microcapsules, and nanoparticles, *etc.* The characteristic biopharmaceutical use of LBG is in oral delivery system (ODS) formulation, while there are reports on its application in buccal, topical, ocular, and colonic delivery and these are discussed in the sub-sections. In 1936, LBG was the first galactomannan made known as an inexpensive substitute for gum tragacanth,⁷⁰ and for various purposes, LBG was rapidly incorporated into the formulations; as a suspending, thickening, and stabilizing excipient in oral and topical liquid dosage forms, especially emulsions.⁷¹ The general applications of LBG are shown in [Figure 9.3](#). Besides its advantage toward human health LBG have the appropriate physicochemical characteristics such as biocompatibility, bioabsorbability, biodegradability, non-teratogenic and non-mutagenic, and the degraded products are excreted readily,⁷² which attract more and more researchers. For controlled delivery, mainly systemic/local delivery of biologically active agents, LBG can be utilized as bioadhesive polymers. Hence, in the biopharmaceutical industry, more and more applications of LBG have been explored⁷³ ([Table 9.1](#)).

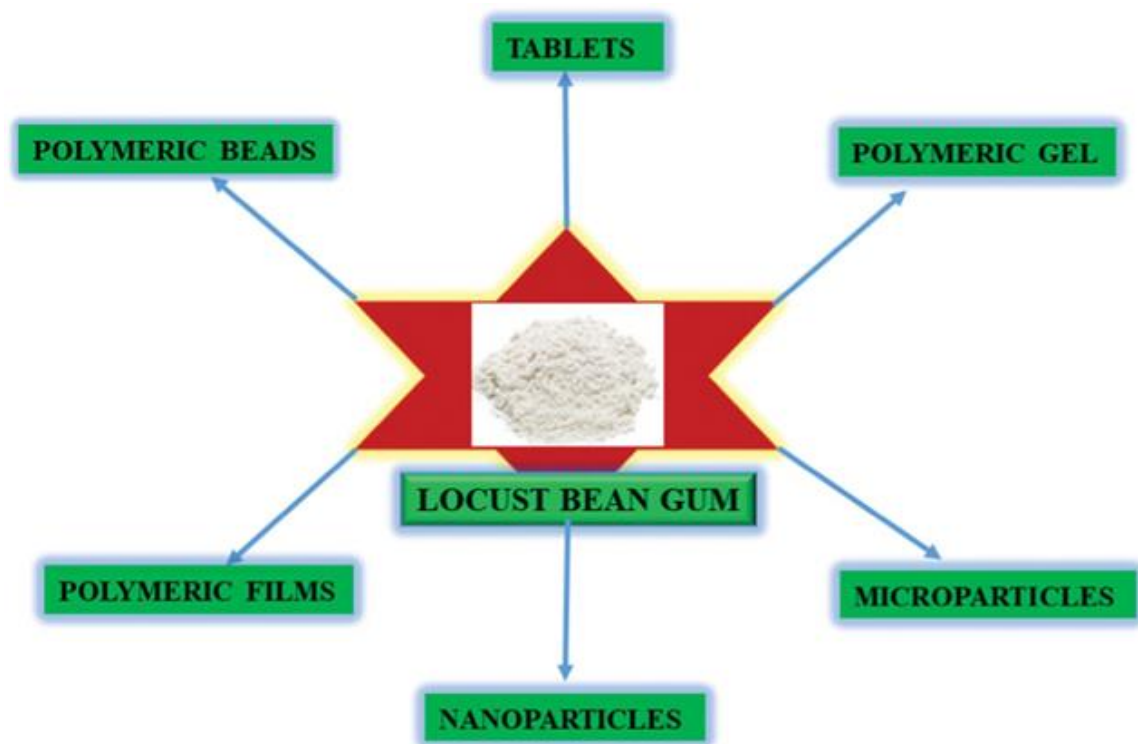


Figure 9.3 Drug delivery applications of LBG.

Table 9.1 LBG in drug formulations.

Gum polysaccharide	Drug	Formulation	Application
Locust bean gum	Captopril	Beads	Gastro retentive delivery ⁷⁴
Locust bean gum	Aceclofenac	IPN nanocomposites	Controlled drug delivery ⁷⁵
Natural locust bean gum	Glipizide	Hydrogel beads	Controlled drug delivery ³⁸
Natural locust bean gum	Diclofenac sodium	Interpenetrating network (IPN) nanocomposites	Enhance the drug release and reduce gastric side effects ⁷⁶
Natural locust bean gum	Buflomedil hydrochloride	Microspheres	For controlled oral drug delivery ⁷⁷

Natural polymers locust bean gum	Capecitabine	IPN microbeads	Sustained/extended drug delivery ⁷⁸
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9.6.1 Oral Drug Delivery

The distribution of a drug *via* oral drug delivery (ODD) is ideal; since it signifies the minor invasive way in formulations of the drug, and the drug excipients should satisfy diverse necessities. As for filling materials, these are applied to enhance the solubility of the active drug and its bioavailability. This will facilitate the development of a dosage form, and the final formulation attains a specific release profile or increase the final drug's product stability.⁷⁹ Polysaccharides suggest a dynamic assembly of excipients. In oral administration methods, polysaccharides are usually considered a major part of drug release from the matrix.

In ODD, LBG is used as a matrix-forming material.⁷³ LBG is generally considered to offer efficient absorption of the drug, controlling drug release from the matrix due to its good swelling capacity. The best typical biopharmaceutical use of LBG is in the formulation of ODD. Sujjaareevath *et al.* (1998) studied the use of LBG formulations of tablets as a sole polysaccharide excipient.⁸⁰ The study confirmed that LBG could cause a reduction in the release of drugs and the rate of erosion and kinetics of drug release. There are reports regarding the application of LBG as a disintegrant, and the results indicated that in the oral dispersible tablet, LBG had a strong disintegration ability. Two latest studies showed that in orodispersible tablets, LBG acted as a superdisintegrant. Nimesulide (a nonsteroidal anti-inflammatory drug) tablet containing 10% LBG produced a 13 s disintegration time. The time for disintegration is folded when crosscarmellose sodium, a known superdisintegrant, was applied an alternative to LBG.¹⁹ The second study is about the preparation of orodispersible tablets using LBG comprised ofloxacin-loaded Eudragit[®] microspheres. Between a concentration of 2.5 and 10%, LBG was used for the study, and the time of disintegration changed between 12 and 20 s. To mask its bitter taste, Ofloxacin was initially encapsulated in microspheres.⁸¹

A novel polysaccharide-based control release matrix technology was designed from an LBG/xanthan gum combination called TIMERx.⁸² An *in vitro* controlled release profile and *in vivo* release profile showed great synergy between polymers TIMERx.⁸³ This technology is commercially accessible nowadays from Penwest Pharmaceuticals and was initially established for oxymorphone dosing twice a day in patients with medium to acute pain. A whole evaluation of uses of TIMERx has been reported by Staniforth and Baichwal.⁸⁴ Therefore, in ODD, LBG has wide applications.

To increase the solubility of the drug lovastatin, LBG, and lovastatin, solid dispersions were formulated. About 53% of Lovastatin was released from the original solid dispersion of LBG and 65% from treated LBG solid dispersions. Among the different solid dispersion methods tested the modified solvent evaporation method confirmed good effects, followed by spray-drying.⁸⁵ There are reports on LBG/xanthan gum to prepare the hydrogel for the controlled release of prednisolone. A reduced release rate of drug from the hydrogel happens when there is an increase in gum concentration, suggesting the diffusion of the drug is controlled by the three-dimensional network density present in the matrix structure. As hydrogel additives, glycerin and sucrose were used to significantly decrease the drug release rate.⁸⁶

Glipizide (oral hypoglycemic agent), a commonly approved drug for type 2 diabetes mellitus patients, and the glipizide-loaded beads were developed using a carboxymethyl derivative of LBG with aluminum chloride (AlCl_3). Glipizide is a model candidate for incorporation studies of drugs, and for the controlled oral delivery of glipizide the carboxymethyl locust bean beads act as a potential carrier. Besides, *in vivo* anti-diabetic activity of these beads on animal models showed a hypoglycemic effect.³⁸ Jana *et al.* reported the synthesis of alginate LBG microspheres in the presence of calcium ions by ionotropic gelation technique, for the sustained release of aceclofenac in ODD application.⁸⁷ There was a sustained release of aceclofenac over 8 h by *in vitro* dissolution studies. The *in vivo* studies showed anti-inflammatory activity in carrageenan-induced rats after oral administration. The results revealed the capacity of prepared microspheres with sustained aceclofenac release was considered a promising DDS.⁸⁷ To provide immunoadjuvant therapy using LBG and chitosan, Braz *et al.*,

developed LBG-based nanoparticles with sulfate derivatives and formed complexes with chitosan through polyelectrolyte complexation. The synthesized nanoparticles were suggested as adjuvants in oral vaccination. The size of the nanoparticles was about 180–200 nm, and the cytotoxic studies in Caco-2 cells reported no changes in cell metabolic activity. Moreover, and as expected, nanoparticles could induce a systemic response and a response at the mucosal level.⁸⁸

Myoglobin-loaded LBG/xanthan gum hydrogels were freeze-dried for the production of tablets. The two-gum ratio was 1/9, and by 24 h around 44% of the drug was released in water.⁸⁹ Thus the drug release nature was directed by LBG, which prevents the diffusion of the drug from the matrix. A diverse method for the manufacture of tablets with a multi-layered matrix also provides a modified release profile of the drug. Upon tablet development, these DD devices consist of an active solute matrix core with one or more combined modulating layers. The hydration rate of the matrix core and the modulating layers restricts the surface area accessible for drug diffusion.^{90,91} Sodium diclofenac tablets containing LBG, a xanthan gum matrix, or a combination (1 : 1) were made, and in 12 h, they could release more than 90% of the drug; the fastest release is the drug with LBG. For the controlled diclofenac release from the gum matrix, the carboxymethyl cellulose incorporation functions as a triple external layer and have to perform as a release retardant for the hydrophilic matrix core, mainly $\approx 70\%$ for LBG core tablets.⁹²

9.6.2 Buccal Drug Delivery

Buccal drug delivery (BDD) has been reported as a substitute to oral dosing for the degradation in the gastrointestinal (GI) tract or hepatic metabolism. BDD compromises a nontoxic approach of utilization of drugs in case of toxicity. Meanwhile polymers obtained from natural sources are found in plenty, are harmless and nontoxic, and they are considered a good candidate for the BDD. Through the buccal mucosa, the drug administration promises two significant benefits (a) preventing pre-systemic removal inside the GI tract and (b) a first-pass hepatic effect.^{93,94} The BDD can be enhanced by ameliorating the inadequately absorbable drug bioavailability in the intestinal area.⁹⁵ As LBG has a mucoadhesive profile with strong

mucoadhesiveness the BDD can be possible. There are not many reports regarding the application of LBG for BDD. LBG-containing tablets or a combination of LBG/xanthan gum was prepared to increase the metoprolol bioavailability by preventing an overall first-pass effect. The tablet formulation requires xanthan gum/LBG combinations that show more effective physical integrity, hardness, and mucoadhesion strength. In 45 min, 2 : 1 LBG/xanthan gum tablets showed complete drug release, and 1% sodium lauryl sulfate was added to the preparation resulting in better drug permeation through porcine buccal mucosa.⁹⁶ There are reports on tablets of LBG/chitosan with ratios of 2/3, 3/2, and 4/1 given to human subjects by comparing propranolol hydrochloride bioavailability. Indeed, greater mucoadhesion was reported for tablets with a ratio 2/3, because of the utmost content of chitosan and showing the whole release *i.e.*, in 10 h a release of 98%, as related to 92% and 90% of formulations with ratios of 3/2 and 4/1 LBG/chitosan, with not many significant changes.⁹⁷ Additionally, in 16 healthy human volunteers the bioavailability study was executed. The maximum bioavailability was shown by the 2 : 3 LBG to chitosan ratio formulation. Hence the work specified that with chitosan, the LBG exhibited a mucoadhesive property for buccal tablets.⁹⁷

9.6.3 Colonic Drug Delivery

Colonic drug delivery (CDD) has increased the attention not only for delivering the drugs for the treatment of local diseases associated with the colon but also for its potential for the delivery of proteins and therapeutic peptides. For systemic absorption of drugs, CDD is also helpful, mainly for protein and peptide drugs, due to the less hostile atmosphere in the colon than in the stomach and small intestine. Targeting drugs orally administered to the colon include coating with pH-dependent polymers, designing time-release dosage forms, and utilizing carriers that are degraded by colonic bacteria.⁹⁸

LBG, as a natural polysaccharide, is a suitable polymer for targeting the drugs to the colon. They can be easily modified chemically and biochemically, and are highly stable, safe, non-toxic, hydrophilic, gel-forming, and biodegradable. The colonic micro-flora can break down the polysaccharides into simple saccharides. In recent times, the colon has

seemed like a possible site which deals with distinctive advantages due to its near-neutral pH. In addition, the colon possesses a much longer transit time, the digestive enzymatic activity is lower, and they have a greater reaction to absorption enhancers. A range of colonic bacteria secretes massive quantities of enzymes for digesting polysaccharides in the colon (*e.g.*, β -d-glucosidase, β -d-galactosidase, amylase, pectinase, xylase, dextranase, *etc.*).^{99,100} In addition, the degradation of LBG *in vivo* is ensured by β -mannanase and alternative enzymes existing in the colon.¹⁰¹ For effective colon-targeted DD, the drug should be safe from absorption and then soon released into the colon.

The initial report on CDD uses LBG to produce LBG films cross-linked with butanediol diglycidylether used in theophylline tablet coating. A swelling ability (300–500%) was shown by the LBG films and undertook colonic microflora degradation, enhancing its use in CDD. However, in higher coating amounts, the film mechanical instability was detected, and thus signifies that they are not suitable as colonic drug carriers.¹⁰² The colon-specific DDS based on LBG/chitosan in the ratio of 2 : 3, 3 : 2, and 4 : 1 was assessed and applied as a coating over mesalazine core tablets. The physiological environment of the stomach and small intestine LBG/chitosan mixtures targets drug protection and allows degradation using colonic bacterial enzymes to the coating materials and permits the release of the drug. The results concluded that the drug targeting the colon, mixtures of LBG/chitosan in the ratio 4 : 1 with greater bioavailability and a good dissolution curve is a potential drug carrier.¹⁰³ Dissolution studies on cross-linked LBG coated theophylline tablets were reported by Hirsch *et al.* (1999) and showed that in the dissolution media, these coatings have no mechanical instability, thus signifying that such films are not suitable as colon carriers.¹⁰⁴ Sirisha *et al.* reported the development of LBG microspheres by an ionic gelation method and were able to target the release of Cromolyn sodium (mast cell stabilizer) in the colon for the management of colitis.¹⁰⁵

9.6.4 Topical Drug Delivery

Topical formulations for DD are becoming increasingly prevalent in recent times. Formulations of LBG for topical application were also defined in

many studies. Coviello *et al.* prepared a 1/1 ratio of LBG/xanthan gum hydrogel to encapsulate niosomes loading ammonium glycyrrhizate as a model drug.¹⁰⁶ Niosomes are vesicular nanocarriers and are self-assembled by the hydration of synthetic surfactants and appropriate amounts of cholesterol or other amphiphilic molecules. These are surfactant vesicles that are non-ionic, over conventional liposomes with numerous benefits, comprising excellent chemical stability, low cost, and greater accessibility of materials.^{107,108} The study reported that LBG/xanthan gum gels are stable and appropriate for topical applications; moreover, they do not need to add any preservatives after one year. Carafa *et al.*, in their study, reported that LBG/xanthan (1 : 1) was used to entrap the niosomes, and drugs, like calcein, ibuprofen, and caffeine were loaded into the niosomes. The encapsulation of niosomes to the hydrogel protects vesicle integrity and a gentle drug release up to 50 h from the polysaccharide arrangement.¹⁰⁹

9.6.5 Ocular Drug Delivery

Designing a DD system to target a particular tissue of the eye has become a significant challenge for scientists in the field. Ocular drug delivery is an exciting attempt owing to the unique anatomical and physiological barriers present. The use of LBG in the different ophthalmic formulations is safe and releases the drug in a controlled manner.¹¹⁰ LBG gel (1–2.5%) was indicated as a viscosity modifier and augmented the bioavailability. Ophthalmic solutions of LBG with echothiophate iodide decreased the systemic absorption of the drug and improved its topical bioavailability.¹¹¹ Microparticles of LBG/*i*-carrageenan encapsulate gentamicin by emulsification, were additionally incorporated into a polyvinyl alcohol gel that can be used on the ocular surface. An early release in the first 6 h occurs for formulations without LBG; with 10% LBG addition, the release is reduced by over 50%.¹¹² No more reports are available on this method to agree a more comprehensive idea of its prospective—one study reported the treatment of glaucoma niosomal gels loaded with cholinergic drug; pilocarpine HCl was used. Various nonionic surfactants with cholesterol in different proportions by an ether injection method were used to prepare pilocarpine HCl niosomes, and into carbopol 934 and LBG-based gels, these preparations were incorporated. The results shown in the study

confirmed that for the controlled delivery of pilocarpine HCl in glaucoma treatment niosomes containing gels are a favorable ocular carrier.¹¹³

9.6.6 Inhalable LBG Microparticles

In recent times, LBG microparticles have been reported for pulmonary delivery by incorporating first-line antitubercular drugs rifabutin (RFB) and isoniazid (INH). The LBG microparticles revealed a high affinity for macrophages and specified the potential of the microparticulate system for tuberculosis therapy.¹¹⁴ Rodrigues *et al.* reported LBG microparticles for lung delivery applications with a combination of the above-mentioned antitubercular drugs isoniazid and rifabutin as inhalable carriers for tuberculosis therapy. The release of drugs from the microparticulate system was fast in the above study and could reach the alveolar target zone to maximize macrophage uptake.¹¹⁵ There are reports that Diltiazem hydrochloride-loaded modified LBG microparticles can be prepared by an emulsification process for the controlled release of the drug. The results of the work concluded that the LBG microparticles validate the development of controlled DDS for many water-soluble medicines.¹¹⁶

9.6.7 Solubility Improvement of Poorly Water-soluble Drugs

The solubility of lipophilic drugs can be increased with LBG. For example, to enhance the solubility of lovastatin, a poorly water-soluble drug by the method of solid dispersion using modified LBG was used as a carrier. By heating, LBG can be modified with an irreversible decrease in viscosity and without changing the swelling property. The lovastatin solubility increases with increases in the concentration of modified LBG. The dissolution study data reveals that improving the solubility of lovastatin solvent evaporation is an efficient method. The *in vivo* study exhibited a substantial reduction in the activity of HMG Co-A reductase and compared to lovastatin alone an upsurge in solid dispersion of lovastatin. Therefore, for augmenting the bioavailability of lovastatin and for improving the dissolution rate of lovastatin, modified LBG can be accepted as a carrier.^{117,118}

9.6.8 Tissue Engineering Application of LBG

Tissue engineering offers a cellular combination, acellular biomaterials, drugs, genes, or gene products that may be designed, specified, fabricated, and delivered simultaneously or sequentially as therapeutic agents.¹¹⁹ Tissue scaffolds are unique for the major tissue applications of tissue engineering. They are a highly porous interconnected matrix with a three-dimensional structure that acts as a scaffold in cell seeding to restore tissues, healing, or remodeling. They are naturally derived or synthetic. Depending on their shelf-life, the scaffolds of tissues are categorized into two types, *i.e.*, implants which are permanent and temporary. The permanent implants of scaffolds hold their form and strength through the organ regeneration/repair, while with the rejuvenation of the organ or tissue, the temporary scaffolds degrade over time. As a natural scaffold, several galactose-based polysaccharides are used. For the production of multidirectional scaffolds, LBG is used primarily.¹²⁰ An LBG suspension was incorporated into the capsule and is lyophilized. The resultant scaffold was about 1–10 mm thick, and upon drying, it is opaque, and when wet, it is opaque with 2–20 mm thickness. LBG scaffolds can also be synthesized by unidirectional freezing axial, radial, log methods. Thus, the LBG scaffold can be designed for its growth and bioactivity, and the cultured cell can be inserted within it.¹¹⁸

9.7 Conclusions

LBG, as a novel and versatile galactomannan, has extensive application in the area of unique DDS as rate controlling excipients. As inert substances, excipients have been included in drug formulations, which aids the manufacturing process. The efficacy of LBG is validated concerning its gelling capacity, stability, thickening ability, and synergistic effects on other gums/polymers such as *k*-carrageenan and xanthan gum. Many researchers have studied the novel uses of LBG in various DDS. A sustained-release behavior has been shown by LBG-based formulations and the leading applications are in oral drug delivery, buccal drug delivery, and CDD; and the ocular and topical applications have also been defined. Even though controlled DDS based on LBG is still in the growth stage, other LBG-based DDS will appear shortly to enrich the LBG-based formulations concerning

their use. Thus LBG galactomannan will become more significant in the future.

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Chapter 10

Pectin-based Vehicles for Delivery of Therapeutics

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10.1 Introduction

Pectin is an FDA-approved, non-toxic, degradable polysaccharide composed of α -(1–4)-linked D-polygalacturonic acid residues. It is one of the main constituents of the plant's cell wall that serves as the hydrating and cementing agent for the cellulose network. The presence of pectin helps maintain the stiffness and structural integrity of cells, protecting the cell from external stress factors from the environment.¹ It is obtained from various sources like passion fruit peel, jackfruit peels, pomegranate peel, pistachio green hulls, and tomato *via* extraction techniques. Apple pomaces

and citrus peels are the most popular sources of commercially available pectin.² It is also one of the principal compounds of citrus fruits, mango, and apple.^{3,4} Plenty of residues are produced from the extraction of sugar; the most pertinent is the sugar from beet pulp acting as a rich source of the pectin.⁵ They are a complex family of heteropolysaccharides that make up a significant portion of the primary cell walls of dicotyledonous plants and are required for their growth and development.^{6,7}

The chemical structure of pectin highly depends on the plant source.⁸ Chemically, pectin can be classified as low and high methoxyl pectin based on the degree of methyl esters present in the structure, which also imparts different physicochemical properties.⁹ Also, pectin belongs to a heterogeneous group of polysaccharides consisting of an abundant amount of galacturonic acid. It is composed of α -galacturonic acid residues linked through α -(1–4) linkages, consisting of rhamnogalacturonan and homogalacturonan, along with seventeen kinds of monosaccharide units.¹⁰ Based on molecular weights, pectin has been categorized into two classes; viz. high molecular weight or natural pectin, and low molecular weight or modified pectin. The molecular structure of pectin varies depending on its source and extraction procedure, mainly of three types; viz. homogalacturonan ($\sim 65\%$), rhamno-galactone-I ($\sim 25\%$), and substituted galacturonic ($\sim 10\%$).¹¹

It is used in drug delivery systems to decrease cholesterol and scaffolds and hydrogel manufacturing.^{12–14} The compositional and structural features endow pectin with good gelling properties and interact with various small molecule therapeutic drugs and genes, making it an attractive substrate for biomedical applications. Furthermore, the degradation of pectin by pectinolytic enzymes produced by the gut microflora in the colon^{8,15} along with the ability to evade absorption in the bloodstream, enables its application in several disorders like reduction in cholesterol level, serum glucose level, anti-cancer activities through induction of apoptosis in human adenocarcinoma cells, gut inflammation, immunomodulation, drug/nutrient interactions, promoting gut health, and removal of radioisotope and heavy metal ions from the body.^{16–18}

Moreover, pectin is listed as a generally recognized as safe (GRAS) material, which has encouraged its wide usage in the pharmaceutical field

for developing various pectin-based formulations (nanoparticles, microspheres, nanofibers, microneedle patches, and hydrogels) for drug delivery, gene delivery, wound healing, and tissue engineering. In this chapter, we have highlighted the distinct properties of pectin, and its application as vehicles for the delivery of therapeutic agents *via* different routes of administration. Furthermore, we also discuss current clinical progress regarding pectin-based vehicles and highlight the hurdles that need to be overcome.

10.2 Overview of Pectin as a Biomaterial

Pectin is primarily used in the food industry and pharmaceutical industry in thickeners, stabilizers, emulsifiers, and gelling agents.^{9,19} However, its excellent biocompatibility and controlled biodegradation with non-toxic degradation products make pectin an appealing biomaterials platform for therapeutic delivery applications, as shown in [Figure 10.1](#).²⁰ Pectin is considered a non-toxic polysaccharide because it cannot be digested by enzymes present in gastric or intestinal regions but is entirely metabolized by colon gut microflora pectinolytic enzymes.²¹ As pectin cannot be absorbed into systemic circulation, it cannot directly affect the body systems and is considered a safe material for oral consumption.²² Also, pectin is soluble in environment-friendly (green) solvents like water,²³ and results in the formation of a gel when subjected to a specific quantity of cations (divalent) (example: calcium (Ca^{+})).^{23,24}

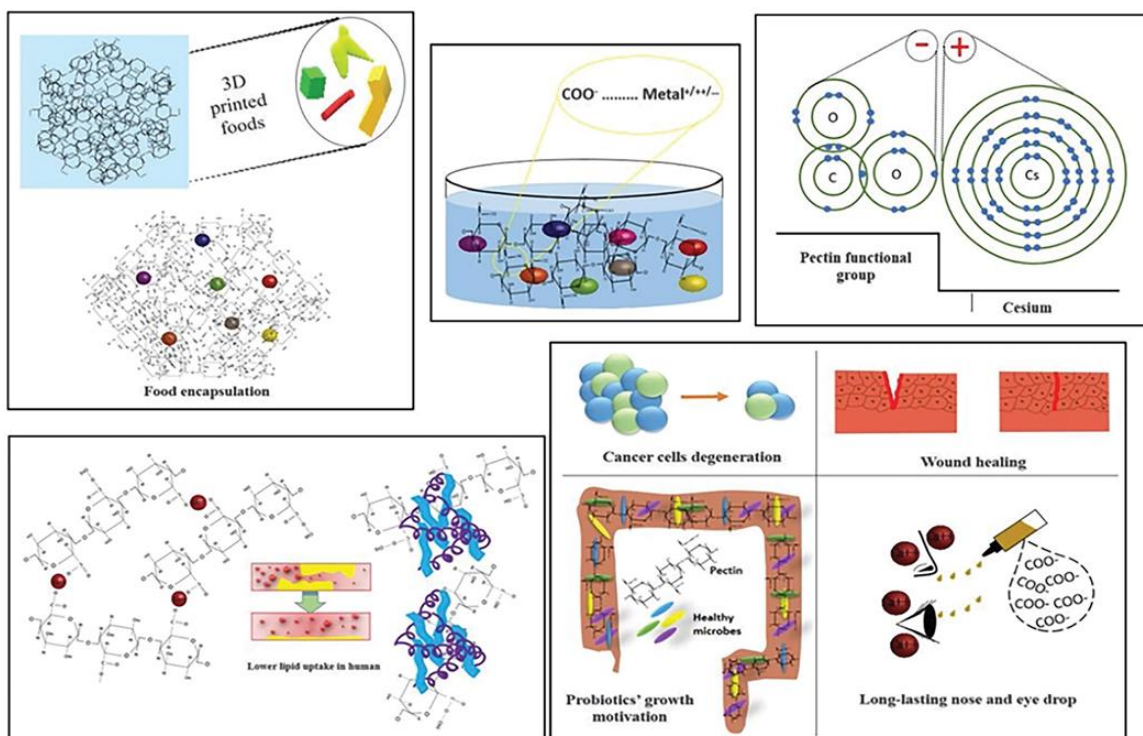


Figure 10.1 Beneficial applications of pectin in different scientific areas. Reproduced from ref. 41 with permission from Elsevier, Copyright 2021.

Additionally, depending on the application, the physicochemical properties of pectin can be tuned.^{20,24} Pectin has been extensively investigated for various therapeutic delivery methods and tissue engineering of skin,^{25,26} bone,²⁷ and cartilages.^{28,29} Pectin, on the other hand, has a relatively low level of protein adsorption and cell adhesion. Pectin was combined with various polymers and peptide sequences for these purposes in several experiments.^{20,27,30}

The degree of esterification (DE) in the pectin structure is known to play a vital role in its ability to be used in the form of an emulsifier, gelling agent, and stabilizer.³¹ Generally, pectin is known as low methoxyl pectin (LMP) due to a low degree of esterification (< 50%) and high methoxyl pectin (HMP) due to a high degree of esterification (>50%). In contrast to HMP, which is less electronegative and strongly hydrophobic, LMP is more electronegative.³² LMP pectin can form three-dimensional gels by interacting chemically with polymers (opposite or neutral charged) and

electrostatic or hydrophobic interactions with cross-linkers.²⁴ The exchange of pectin's carboxymethyl groups with amine-containing molecules improves pectin's functionality, such as increased water solubility.³³ Several studies comparing the stabilizing ability of LMP and HMP in liposomes have been performed. For example, resveratrol liposomes prepared using LMP (30% DE) can protect liposomes from several environmental parameters like ionic strength, pH, and temperature more efficiently than HMP (70% DE).³² According to another study, vitamin C liposomes coated with LMP had more excellent stability and skin penetration potential than coated HMP.³⁴ The better ability of the LMP can be corroborated by the availability of more quantities of carboxyl groups, which produces a negative charge on liposomes and increases the electrostatic repulsion among vesicles. It also results in better binding of LMP to the surface *via* hydrogen bonding. As a result, LMP is the most popular liposome coating material for polyphenolic phlorizin³⁵ and collagen peptides like bioactive to improve their stability and bioavailability.³⁶

Several pectin-based formulations have been utilized to manage digestive system (including dysbiosis) and gastrointestinal tract disorders (GIT). As a result, pectin promotes motility and intestinal peristalsis while slowing food absorption, cleaning the small intestine villi, and enhancing the absorption of active pharmaceutical compounds. Furthermore, pectin is also used to prevent and manage a hypoglycemic condition, atherosclerosis and metabolic disorders (obesity, diabetic Mellitus), and liver and pancreas-related diseases.^{18,37–40}

10.3 Variety of Pectin-based Delivery Vehicles

Recently, pectin has received increased interest in the development of various therapeutic delivery platforms such as particles, hydrogels, and fibers.⁴² Pectin forms a viscous solution in the presence of divalent cations and acts as a mucoadhesive biopolymer.⁴³ It is non-toxic⁴⁴ and has low production costs⁴⁵ and high bioavailability.⁴⁶ Most importantly, it can aid in delivering drugs through many routes like oral, nasal, vaginal, ocular, gastric, and especially those in the large intestine.^{47,48}

Several pectin-based formulations have already been explored, like hydrogels, microspheres, films, and nanoparticles.⁴⁹ Catori *et al.* have prepared pectin and nanocrystal-based hydrogels to understand hydroxyapatite nucleation and growth using the biomimetic method. Its efficacy was analyzed on the fibroblast L929 cell line in terms of cell viability. A comparison study was carried out between hydrogels prepared by a biomimetic and chemical precipitation method. Later on, it was concluded that hydrogels prepared by biomimetic methods were superior to that of chemical precipitation incompatibility.⁵⁰ Similarly, pectin was also utilized to formulate nanoparticles; for this, Ergin *et al.* prepared pectin nanoparticles encapsulated in microparticles to achieve the synergistic effect and better bioavailability and target specific drug delivery for colon targeting by using S-adenosyl-L-methionine. *In vivo* study in Wistar rats revealed that the S-adenosyl-L-methionine loaded pectin nanoparticles showed higher bioavailability than the only S-adenosyl-L-methionine loaded solution upon oral administration and showed a better therapeutic effect in colon targeting.⁵¹ Similarly, Cui *et al.* fabricated 3D scaffold nanocomposites by freezing the drying process for bone tissue engineering. In this gallium-apatite/chitin/pectin nanocomposite, varying concentrations of nanocomposites were prepared, and then analyzed using various techniques such as FTIR and XRD. This analysis proved that the prepared scaffold was biocompatible, highly porous, and mechanically stable. Furthermore, its efficacy was also analyzed on NIH3T3 and MG-63 cells, for which no toxicity and cell attachment were observed. Nižić *et al.* developed pectin/hypromellose-based microspheres using the spray drying method for the nasal delivery of melatonin. The varying ratio of hypromellose was analyzed for efficient nasal delivery, which showed better entrapment and melatonin dissolution compared with pure melatonin. Pure melatonin was conjugated with pectin, which showed better melatonin nasal delivery and higher swelling ability and mucoadhesive properties. *In vitro* deposition study was carried out using a transparent silicone nasal cavity model that revealed better therapeutic potential.⁵² On the other hand, Vaidya *et al.* prepared a metronidazole-loaded pectin microsphere for colon delivery. Microspheres were synthesized using the emulsion dehydration technique. The synthesized metronidazole-conjugated pectin showed better

drug entrapment efficiency, and no drug release was observed from the microsphere at acidic pH at the gastrointestinal tract. *In vivo* study in albino rats revealed that the formulation was accessed to various parts of the GIT and the colon in varying concentrations at different intervals of time. Hence, it concluded that metronidazole-conjugated with pectin delivers the drug more efficiently to the colon.⁵³ Furthermore, pectin has also been used for developing nanofibers using the electrospinning technique.⁵⁴ Alipour *et al.* developed silver nanoparticle-loaded nanofibers of pectin blended with polyvinyl alcohol, polyvinylpyrrolidone, and mafenide acetate. It is an *in vitro* effect; the antibacterial activity was checked against *E. coli*, *S. aureus*, and *P. aeruginosa*, and a cytotoxicity assay was carried out to determine the cytotoxicity and biodegradability. Additionally, an *in vivo* study in white rabbits revealed dermal regeneration and effectiveness in wound healing.⁵⁵

10.4 Pectin as a Suitable Mucoadhesive Vehicle

The development of mucoadhesive and bioadhesive delivery systems for administering the drug through one or more mucosal surfaces of the body is one of the advanced drug delivery and therapeutics areas. The availability of a more significant network of blood vessels and higher surface area makes transmucosal routes preferable for both local and systemic drug delivery. Moreover, they offer benefits like bypassing the first-pass hepatic metabolism and drug degradation inside the stomach's acidic environment, thus delivering the drug directly to the bloodstream, in turn, giving augmented bioavailability.⁵⁶

So far, the transmucosal surfaces used for investigating their potential for administering drugs to get therapeutic effects include oral (sublingual and buccal),⁵⁷ nasal,⁵⁸ ocular,⁵⁹ wound⁶⁰ and vaginal surfaces.⁶¹ In addition to their classic advantages of bypassing the first-pass metabolism and pain associated with oral and parenteral administration routes, respectively, they are of great significance when a drug is required at a local site in limited doses, for instance, in local infections demanding antibiotics.^{62,63}

To achieve effective mucosal administration, the delivery vehicle/matrix has to meet specific attributes, prominent of which are biocompatibility over an extended range of applications, bioadhesiveness (mucoadhesive),

biodegradability, and ease of processing while developing varied dosage forms. Most novel drug delivery systems for the purposes mentioned above are prepared using synthetic or biomaterial-based polymers. However, due to their well-established biocompatibility and biodegradability, natural biomaterials are often used, and they extensively serve the purpose. In addition, almost all of them exhibit bioadhesiveness. One such prominent group of natural biomaterials is polysaccharide-based polymers, which range from prevalent materials like starch to quite complex examples like pectins, sodium alginates, and chitosan derived from diverse natural sources or in semi-synthetic form.⁶⁴

For decades, pectin has been used in the pharma sector as an efficient gelling agent; however, for the past few years, its use in developing controlled drug delivery systems has prevailed the former usage.^{45,65,66} This might be corroborated by their long-standing reputation of being safe and non-toxic and hence the inclusion in the GRAS (generally regarded as safe) category of the US FDA.^{42,44,66} Also, they incur low production costs⁴⁵ and are readily available.⁴⁶ Because pectin is well tolerated by patients,^{42,66,67} it has been extensively explored in delivering an array of drugs *via* oral, nasal, and vaginal routes.^{42,66,68–73}

The oral route is undoubtedly the most preferred route of drug administration in general because it requires less effort, minor pain, the highest degree of convenience, extreme patient compliance, and insignificant infection risk compared to the subcutaneous route of administration.⁶⁷ On the other hand, several limitations are also associated with this administration route, such as fast protein and polypeptide degradation *via* digestive enzymes action in the gastrointestinal tract, reasonably low absorption of actives across the mucosal epithelium resulting in low bioavailability, acidic degradation, and enzymatic proteolysis of drugs inside stomach post oral administration.^{42,66} Several strategies have been escalated and adopted to augment the buccal penetration, some notable examples of which are the use of protease inhibitors,⁷⁴ permeation enhancers,⁷⁵ enteric coatings,⁷⁶ and biopolymer-based micro and nanosphere formulations.^{77,78} On the contrary, protein drugs are fundamentally not prone to such acidic degradation and enzyme-mediated proteolysis in the colon region of the gastrointestinal tract; hence,

concentrated efforts for their colonic delivery have attained great interest.^{66,71,79} Thus, various polymers, including pectins, have been acknowledged as shielding agents against enzymatic proteolysis.⁸⁰ Therefore, pectin-stabilized polypeptide drugs remain intact inside the stomach and small intestine and later post-digestion of pectin by the microflora in the colon; it results in drug molecule release.⁸¹ The degree of pectin susceptibility to enzymatic attack is improved in the presence of calcium ions, whereas it decreases post methyl esterification. The shortcoming of using pectin alone is premature drug release from the system as pectins tend to swell under physiological conditions⁸² (Liu *et al.*, 2006). To minimize this effect, combinations of pectins with other polymers such as acrylic or cellulosic polymers, zein, chitosan, and hydroxypropylmethylcellulose have been predominantly used.^{42,65,81}

The major drawback or lagging point allied to oral drug delivery is its short clearance time across the whole alimentary tract (around 4–12 h), which renders a major part of the administered drug unabsorbed. Thus, in recent times the nasal route has gained dominance in dosage form feasibility and performance over the oral route. Drugs, when administered nasally, get absorbed through nasal epithelium in a relatively shorter time duration of even ~15 min. The other significant glitches associated with the oral route are degradation of protein and peptide-based drugs inside the gastrointestinal tract and lower trans-mucosal permeability. Mucoadhesive and macromolecular carrier-based systems have been explored for quite a lot of years, and amid this period, pectin-based drug delivery systems have emerged as predominantly promising. The traditional use of low methoxyl pectins obtained from fruits, which are polyanionic solid polyuronides of a chemical nature, in the preparation of jellies and jam is well documented in the literature. These pectin polyuronides tend to form weak gels in the presence of Ca^{2+} ions (at a concentration equivalent to 8 meq L^{-1}), which naturally occurs in the nasal secretions.⁸³ Nonetheless, the smooth texture and non-rigidity further increase patient compliance and make them a preferred choice among available nasal delivery formulations.^{67,84}

These gels exhibit a pseudoplastic flow^{68,72,85} and the drug release from them is diffusion and dissolution dependent at low^{42,68} and high⁴² concentrations of pectins, respectively. Additionally, they hold the

incorporated substance inside the nasal cavity for an extended period that aids in modulating its systemic absorption rate. In general, pectins do not directly enhance drug absorption; hence cannot be called an absorption enhancer. However, they lead to the opening of tight junctions and, because of calcium chelation, aid in altering drug release patterns.^{86,87} Also, they possess mucoadhesive strength,^{42,69,72} though not comparable to that of chitosan.⁶⁹ Their mucoadhesive potential is dependent on factors like local mucosal site pH, viscosity, functional groups of pectin, and molecular weight.^{42,72} Besides all these, sample volume restriction in nasal drug delivery (at max ~150 µl can be delivered) limits its implication in delivering those sparingly soluble drugs and those needed to be delivered over an extended period.⁴²

In addition to the nasal mucosa, vaginal mucosa has been proven to be an effective drug delivery and drug absorption site due to its rich blood circulation, higher surface area,⁸⁸ and well-implicated microflora.⁷³ The rates of drug release from a system may vary at different points in the menstrual cycle, and this parameter is crucial when considering drug delivery during menopause.⁷³ Being a tube-like body cavity, most of the vaginal drug delivery systems work based on muco-adhesion mechanisms.^{89,90} The vaginal route has proven to be favorable and quite promising in delivering a range of drugs, including those of great clinical importance such as human growth hormone and propranolol. Furthermore, vaginal delivery of hormones for attaining hormonal contraception has been sought as a comparatively more efficient was compared to the oral route.⁷³ The vaginal route holds many substantial advantages compared to the nasal route, with only one major limitation: this choice is gender limited. The use of pectins as mucoadhesive polymers has been widely explored in a range of vaginal formulations, and owing to pectin's features of the highest swelling volume and mucoadhesive strength with slight alterations in pH; they are gaining more attention and adaptability by formulation scientists while designing vaginal dosage forms.^{91,92}

10.5 Range of Therapeutics Delivered via Pectin Vehicles

10.5.1 Pectin Vehicles for Small Molecular Drug Delivery

Dihydroartemisinin (DHA) is an effective anti-cancer agent due to its high potential, but its low solubility and premature release restrict its use. Developing a DHA-encapsulated system that allows for targeted identification and precise sustained release has resulted in opportunities and challenges that an endogenous stimulus can activate. As a result, a dual-targeted DHA containing nanocarrier was formulated by combining the advantages of increased protein with galactose residue expression (asialoglycoprotein receptor) on the liver's surface and high folic acid (FA) receptor expression. To make folic acid-pectin-eight-arm polyethylene glycol dihydro-artemisinin prodrugs (FA-Pectin-8armPEG-DHA), researchers attached DHA-loaded eight-arm polyethylene conjugates to pectin, then encapsulated hydroxy camptothecin using self-assembly of both (hydrophilic and hydrophobic carriers) to make a folic acid-pectin-eight-arm (FPPDH NPs). FPPDH NPs indicated a particle diameter around 98 nm with drug loading about 7.4% weight and entrapment efficiency around 20.57% weight. The developed nanoparticles showed the improved cytotoxicity of 204.5-times (H22) and 178.4-times (HepG2) to the free DHA, respectively. Furthermore, a synergistic effect of compounds demonstrated that dual-targeted combination therapy is a promising treatment for cancer.⁹³

10.5.2 Pectin Vehicles for Gene Delivery

DNA carriers hold much promise for gene therapy. The structure of pectin is composed of galactose residues that act as promising ligands for membrane receptor interaction. For complexation with DNA, anionic pectin was altered with three various amine groups (cationic). Pectin-NH₂ was formulated by replacing the carboxyl groups of galacturonic acids with primary amine groups, which were then modified to create pectin-T (T = N + H(CH₃)₂) and pectin-NH₂-Q (Q = N + (CH₃)₃). Gel electrophoresis revealed that all three altered pectins form complexes with plasmid DNA. Transmission Electron Microscopy (TEM) was used to determine the size and morphology of the pectin-NH₂/DNA complexes. Human embryonic kidney cell lines (HEK293) and plasmid DNA encoding for green

fluorescence protein were used in transfection experiments (GFP). Transinfection potential was determined by flow cytometry analysis using FACS. Pectin–NH₂–Q served as the promising carrier in gene delivery. The addition of chloroquine to the transfection medium increased HEK293 transfection significantly, suggesting that endocytosis is the most probable internalization mechanism, which refers to the endosome complex's inability to escape. By preventing pectin binding to membrane receptors (galectins) with the aid of lactose and galactose as competitive inhibitors to this interaction, pectin galactose residues support transfection. The decrease in transinfection potential demonstrated the role of pectin's galactose residues in HEK293 transfection. Thus, the modified pectin may be used as a non-viral carrier to deliver targeted genes to cancer cells with galactose-binding lectins on their surface.⁹⁴

10.6 Therapeutic Applications of Pectin-based Drug Delivery Vehicles

10.6.1 Oral Delivery

The oral route of administration is one of the most convenient and standard routes of drug administration because of several advantages like higher patient compliance and self-administration. However, there are some disadvantages associated, such as low bioavailability because of poor permeation of drug towards mucosal epithelium, chances of degradation of drugs, especially polypeptide, proteins due to digestive enzymes in the GIT, enzymatic proteolysis, and acidic degradation.^{66,95} Alternatively, several other active ingredients cannot be given through the oral route because of gastric mucosal damage.⁹⁶

To protect the drug from degradation, various approaches have been studied using a permeation enhancer,⁷⁵ a protease inhibitor,⁷⁴ and an enteric coating, which protect the drug from acidic degradation and results in the delivery of the drug to the targeted region.⁷⁶ Before formulating a pectin-based drug, GIT characteristics must be considered, mainly the pH sensitivity, time-dependency, and therapeutic efficacy. The formulation for oral doses should be designed and developed to protect the drug from

environmental conditions and release the drug at the targeted site without comprising the therapeutic effect.

The major problem related to oral pectin formulations is the high water absorbing capacity at normal physiological conditions, *i.e.* it absorbs up to 160 times the weight of the dry gel, which leads to premature drug release.^{97,98} To overcome this weakness, pectin can be cross-linked with other polymers like zein,⁶⁵ acrylic polymers,⁹⁸ chitosan, and hydroxypropyl methylcellulose.⁹⁹

Pectin is widely used for oral formulations, for example, Singh *et al.* developed mesalamine and saccharomyces boulardii-loaded enteric-coated pectin pellets by coating cellulose acetate phthalate using an extrusion–spheronization technique for colonic drug delivery in ulcerative colitis treatment. To establish the kinetics of drug release inside the body an *in vitro* study was carried out in simulated intestinal and colonic fluid for 24 hours – using a dissolution apparatus and rat cecal for both coated and uncoated tablets. In the dissolution data, it was estimated that around 65% of the drug release was observed within 5 hours, but in the case of the coated drug, only 13% of the drug was released, which showed that the coating could prevent the drug from early release thereby imparting a controlled release behavior. In rat cecal, a greater amount of drug was released from uncoated tablets as compared to coated tablets. At the same time, it was estimated that the release of each uncoated and coated formulation boosted the presence of rat cecal medium in simulated colonic fluid and showed a better release profile.

Similarly, to confirm its therapeutic efficacy *in vivo*, the colitis model was developed in male Wistar rats using hapten 2,4,6-trinitrobenzene sulfonic acid.¹⁰⁰ The rats were divided into various groups, and parameters like bodyweight ratio, histological study, glutathione activity, myeloperoxidase and lipid peroxidase, and diarrhea were assessed. The pharmacokinetic study was also determined, which showed that more coated drug reached the systemic circulation than uncoated drugs. This study estimated that the prepared formulation successfully improved the disease condition in rats, which was confirmed by the gain in body weight, decreased levels of myeloperoxidase and lipid peroxidase, and a surge in the level of glutathione.¹⁰¹ Salama *et al.* prepared meclizine loaded chitosan–

pectin buccal nanoparticles for the management of chemotherapy-induced nausea and vomiting. *In vitro* experiments were set using a Franz diffusion cell where sheep buccal mucosa was implied as a barrier layer, and release over time was calculated. It was concluded that the formulations prepared with the anionic mixture of an equal portion of tripolyphosphate showed higher release than the formulation with the higher pectin content. *In vivo* study was done in 32 male albino rats, and emesis was induced by using cyclophosphamide. Significant studies like measurement of motor activity and coordination, measurement of pro-inflammatory cytokines, and histopathological analysis were observed. It was estimated that the developed formulation was helpful in reducing body weight, elevated emesis due to chemotherapy, anorexia, and management of pro-inflammatory mediators and neurotransmitters, and controlled the behavioral and biochemical changes.¹⁰²

10.6.2 Systemic Delivery

Pectin has been employed for formulating carriers for systemic administration of hydrophobic drugs due to its superior hydrophilicity. The biodegradability, biocompatibility, and immunologically inert properties of pectin comply with all the requirements for a suitable drug carrier for clinical advancement.¹⁰³ Pectin displays inherent anti-tumor activity, which stems from the ability to recognize and interfere with the galectin-3 receptors present on various cancer cells. As a result, a wide range of pectin-based nano-formulations has been designed to deliver chemotherapeutic drugs.^{103,104}

Hydrophobic drugs dihydroartemisinin (DHA) and hydroxycamptothecin (HCPT) were simultaneously delivered for synergistic breast cancer treatment using self-assembled pectin nanoparticles. Liu *et al.* synthesized self-assembled pectin-dihydroartemisinin nanoparticles with a particle size ~70 nm, co-encapsulating the hydrophobic drug HCPT. Fluorescence imaging and cytotoxicity evaluation showed enhanced cellular uptake of the pectin nanoparticles by 4T1 breast cancer cells and inhibition of cell viability, respectively. *In vivo* pharmacokinetic assessment of intravenously injected self-assembled nanoparticles in 4T1 bearing mice suggested a 4.8 and 6.7-times higher blood circulation half-time of DHA and HCPT as

compared to free drugs. The pectin formulation helped prolong the blood circulation time of the drugs and reduced the risk of hypersensitive reactions in comparison to free drugs (DHA and HCPT). Mice treated with dual-drug-loaded nanoparticles (PDC-H NPs) demonstrated a marked decrease in tumor volume with no noticeable change in average body weight, unlike mice treated with free DHA and HCPT.¹⁰⁴ Thus, the pectin-based carrier system displayed promising potential for targeted anti-cancer therapy.

Similarly, Liu *et al.* developed HCPT-embedded folate-modified pectin nanoparticles loaded with ursolic acid (UA) for improved synergistic inhibition and elimination of hepatic tumors ([Figure 10.2](#)). The nanoparticles displayed high drug loading and folic acid groups on the surface-enhanced accumulation into liver cancer H22 cells. Pharmacokinetic study of the pectin nanoparticles (HCPT@F-Pt-Pu NPs) administered through tail vein injection into H22 bearing mice showed extended circulation times of up to 80 h as compared to free UA – 7 h and HCPT – 8 h, respectively. The actively targeted pectin nanoparticles ameliorated the rat survival rate by inhibition of tumor growth and showed negligible off-target side effects, giving it great potential as a sustained drug delivery system.¹⁰⁵ Thus, pectin-based formulations have great potential for biomedical applications, mainly for drug delivery. Other pectin-containing carriers that have been developed for intravenous drug delivery have been summarized in [Table 10.1](#).

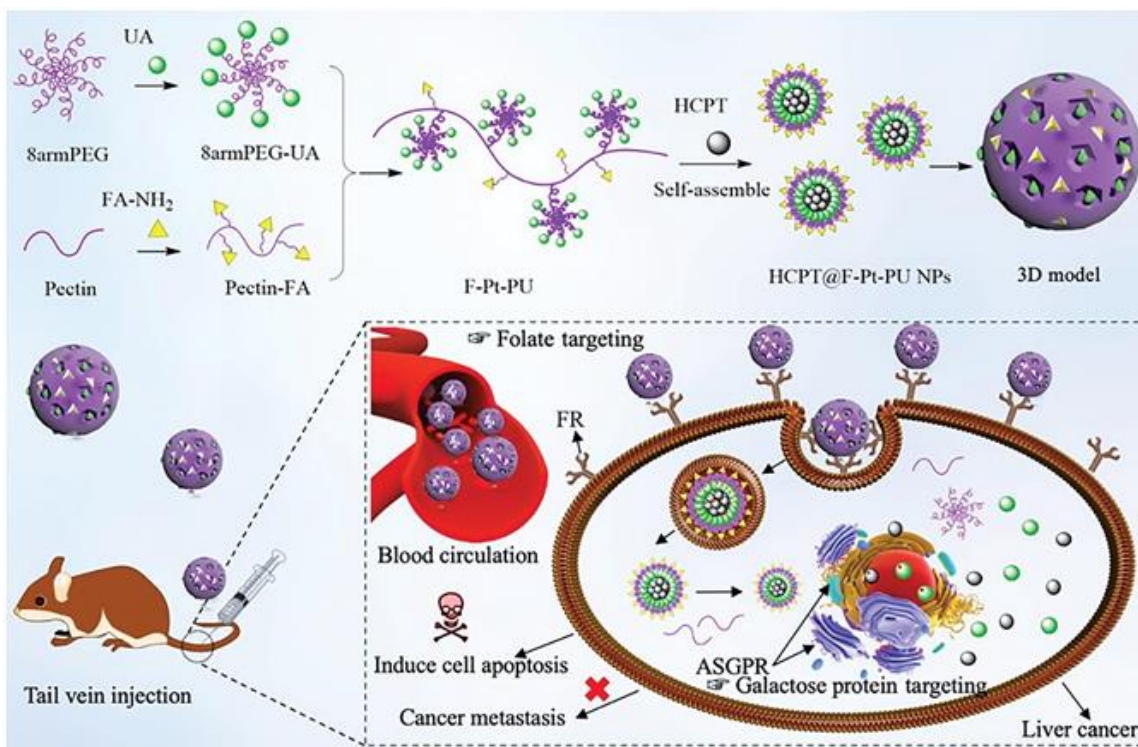


Figure 10.2 Representative image of HCPT@F-Pt-PU NP preparation and intracellular targeted delivery. Reproduced from ref. 105 with permission from America Chemical Society, Copyright 2021.

Table 10.1 The various formulations of pectin.

Sr. n o.	Formulation	Drug	Disease	Ref.
1.	Pectin–silica gel beads	Mesalazine	Colon targeting	143
2.	Core–shell structure pectin–eight-arm polyethylene glycol– ursolic acid/hydroxycamptotheci n nanoparticles	Ursolic acid (UA) and 10- hydroxycamptotheci n	Cancer	144
3.	Amidated pectin microparticles	Sulfasalazine	Inflammatory bowel	145

		disease	
4.	Folic acid-modified nanoparticles	Dihydroartemisinin	Liver cancer 146
5.	Pectin nanoparticles	Fluorouracil (5-FU)	Hepatocellular carcinoma 147
6.	Self-assembled pectin–dox conjugate (PDC-NPs)	Doxorubicin	Hepatocellular carcinoma 148

The inherent gelling property of pectin has been exploited to form *in situ* gelling hydrogels based on an internal ionotropic gelation strategy using $\text{CaCO}_3/\text{d-glucono-}\delta\text{-lactone}$. The prepared hydrogels form attractive substrates for tissue engineering and regenerative medicine as they mimic the extracellular matrices. Neves *et al.* prepared RGD peptide conjugated pectin hydrogels with varying viscoelasticities for cell delivery. hMSC, a promising cell type for regenerative medicine, was embedded into the pectin hydrogel by mixing with the gelling components. The embedded hMSC remained viable and metabolically active for up to 14 days.

Furthermore, they maintained their phenotypic morphology with established cell to cell contact, mimicked the extracellular matrix, and migrated the hydrogels outward. A 7 day preliminary study in mice through subcutaneous implantation of acellular RGD–Pectin hydrogels demonstrated advanced degradation profiles which could be adjusted by varying the polymeric content. The observations from this study manifested the promising ability of pectin to be used as a biomaterial for hydrogel preparation for cell delivery in tissue engineering and regenerative medicine applications.¹⁰⁶

10.6.3 Intranasal Delivery

Current studies have indicated that the nasal route can be utilized to deliver active pharmaceutical compounds (low molecular weight), proteins, peptide molecules delivered *via* injection or when faster drug release is expected. Thus, mucoadhesive polymers have been put forth to manufacture carriers to attain controlled release using the mucosal tissue's principle of hydration/diffusion mechanism. Due to the strong hydrogen bonding between pectin-free acid groups and mucin, LMP pectin has been shown to have mucoadhesive potential in recent studies.¹⁰⁷ Pectin changed from an

aqueous solution to a gel after being applied to mucosal surfaces under suitable conditions. Pectin has been studied for sustained, faster, or pulse delivery *via* nasal routes. Pectin is used for various applications *via* the nasal route. For example, opioid pain killer (Fentanyl)-pectin nasal spray was shown to enhance the analgesic action, pain management associated with cancer, where a faster release of drug is required.^{108–110}

PecSys™ (PS) is a patented pectin-loaded drug delivery device that transforms into a gel after being applied to mucosal areas and has potential applications for drugs used in local and systemic delivery for the treatment of a variety of diseases. Intranasal formulations containing opioid analgesics are at the most advanced drug production stages to provide quick pain relief with simple and safe dosing and fewer side effects. The profile of such drugs means they may not be helpful *via* current routes of administration. Thus, pectin plays a crucial role in improving their pharmacokinetic parameters like peak plasma concentration ($C_{\max}$), which has been indicated in clinical testing. The product developed using pectin is a fentanyl nasal spray (NasalFent®), which has successfully attained a prominent role in phase III clinical trials and was filed in 2009.⁴⁴

10.6.4 Topical Delivery

Skin is used to deliver both local and systemic transportation of drugs having to bypass the first-pass metabolism effect, reduction in fluctuation in the drug plasma level, sustained drug delivery, targeted effect, and patient compliance.^{111–113} The two types of drug delivery systems, *i.e.* topical and transdermal systems, are used to deliver the drug through the skin. The topical is mainly used for local skin infections by following the principle of passive diffusion. Conversely, transdermal drug delivery systems deliver the drugs systemically by penetrating the deeper layer of tissues. If the drug is polar, having a significant molecular weight of above 500 Da restricts the entry *via* the physiological barrier.¹¹⁴

Additionally, lipophilic drugs favor the condition to stay in the subcutaneous region. However, hydrophilic drugs do not impart to do so.¹¹⁵ Therefore, if we aim to deliver the drug through the skin, it should be lipophilic, soluble in both aqueous and lipophilic phases, and have a molecular weight of less than 500 Da.¹¹⁶

Several formulations are designed based on pectin for example, Corrocher *et al.* carried out randomized, double-blind clinical trials on 24 patients with desquamative gingivitis to establish the efficacy and tolerability of 0.1% pectin-based tacrolimus equivalent to 0.2 mg versus 0.5% clobetasol propionate, which is equivalent to 1 mg for 28 days taken once a day. Initially, the signs and symptoms of desquamative gingivitis were recorded on a scale of 0 to 3, *i.e.* (0 = absent, 3 = severe), and the endpoint was established in the same way. Patients were evaluated before and after treatment for four weeks followed by two follow up visits, *i.e.*, 6 and 8 weeks. The patient treated with the tacrolimus group achieved remission of signs and symptoms of desquamation like erythema at 4 and 6 weeks. In the 8th week, the percentage of remission was reduced to 66.7%. However, none of the patients treated with tacrolimus showed any improvement in symptoms compared to those treated with clobetasol. Based upon the results of the clinical trials, it was assumed that the 0.1% pectin-based tacrolimus ointment was more effective than 0.5% clobetasol propionate.¹¹⁷ Pectin has also been used as an effect biomaterial for the treatment of cancer. Gazzi *et al.* prepared imiquimod-loaded pectin-based hydrogel nanocapsules for the treatment of melanoma. To check its cytotoxic effect, SK-MEL-28 cells were used. It was determined that the imiquimod-loaded nanocapsules showed time-dependent cell viability, which was not observed in the case of free imiquimod solution.

Additionally, a permeation study was also carried out, which revealed that polymeric pectin-based imiquimod nanocapsule formulation showed enhanced permeation compared with the only pectin-loaded imiquimod formulation. In general, the prepared formulation showed better efficacy in terms of cell viability and permeability compared with imiquimod solution and imiquimod-loaded pectin.¹¹⁸ Besides this, another set of experiments was carried out for the transdermal delivery of pectin based on asiatic acid hydrogel patches to suppress the parasite and reduce the inflammation caused by *P. berghei* murine malaria. To check its therapeutic efficacy, infected Sprague-Dawley rats were used. In this, chloroquine pectin-based patches and asiatic acid pectin-based patches were applied on the mice's shaved dorsal region, which was infected with *Plasmodium berghei*, after seven days of infection. After the application, studies like hematological

analysis, the effect of pectin hydrogel on inflammation, inflammation in C reactive protein, and severe malaria anemia were analyzed. It was observed that the group which was treated with asiatic acid patches reduced parasitemia readily in a dose-dependent manner; however, the group that was treated with chloroquine-based patch took a longer duration of time to clear the parasitemia.¹¹⁹

10.6.5 Ocular Delivery

The eye of a human being is one of the most challenging organs for drug delivery because of its anatomical and physiological limitations and permeability barriers such as nasolacrimal drainage, corneal epithelium, and dynamics of tears that affect the bioavailability of the ocular formulations.^{120,121} Thus, to attain a concentration in the therapeutic range, frequent administration of formulations is required and, at last, large doses in the case of conventional dosage forms applied in the form of eye drops.¹²²

Majzoob *et al.* synthesized pectin–cysteine conjugates and characterized them for permeation, swelling, and mucoadhesion study. The thiolated pectin was composed of 892.27 ± 68.68 μmol thiol groups per gram of pectin and indicated enhanced *in vitro* adhesion by 5-times controlled swelling with increased permeation unmodified pectin.¹²³

10.6.6 Vaginal Delivery

The vaginal route of drug delivery has long been in use. It offers various benefits over other conventional routes, such as oral and intravenous delivery, to evade gastrointestinal environment degradation and the hepatic first-pass effect.^{124,125} In addition, the sizeable vaginal surface area, well-studied microflora, and dense network of blood vessels make it an exciting site for drug administration for local drug delivery^{88,124} in various conditions such as bacterial, fungal, and protozoal infections, HIV prevention, and delivery of contraceptives as well as for systemic delivery of drugs.¹²⁶

The vagina is regarded as a mucosal tissue, where the mucus coating plays an essential role in various physiological functions but is also

subjected to continuous chemical changes during menstrual cycles and during the self-cleaning process, impeding the effectiveness of drug delivery.^{127,128} The various commercial dosage forms developed for the vaginal application of drugs such as creams, foams, gels, irrigations, and tablets face low retention time in the vaginal cavity, leakage, and less patient compliance due to high dosing frequency.^{73,128} Thus, to circumvent these issues, mucoadhesive polymer-based matrices can be employed to deliver drugs effectively.^{73,127}

Pectin polysaccharide has good bioadhesive properties. Hence new approaches based on pectin have been used to develop vaginal formulations for the delivery of various drugs.⁹¹ Pectin–chitosan based tablet formulations containing anti-HIV drugs have been investigated to prevent sexual transmission of HIV from male-to-female, which is a significant point of concern. The addition of mucoadhesive pectin demonstrated superior mucoadhesive strength and retention time within the vaginal mucosa because the combination of both polymers forms a polyelectrolyte complex, producing a robust gelled system that can control the release of tenofovir, an anti-retroviral drug. The formulations can be regarded as a good option for the self-protection of women from the sexual transmission of HIV.¹²⁹ Bacterial or fungal infections of the vagina are another severe issue characterized by vaginal inflammation, irritation, itching, white to pale white discharge, and discomfort. To address this problem, Mishra *et al.* formulated pectin–chitosan mucoadhesive vaginal films loaded with fluconazole, an anti-candidal drug having no significant side effects, for local administration. The films were prepared using chitosan and pectin in a 75 : 25 ratio and DMSO as a permeation enhancer. The evaluation of the *in vitro* diffusion in stimulated vaginal fluid and *ex vivo* diffusion study in mucosa from rat vaginas showed that the films allowed controlled diffusion of the drug.

Furthermore, the films exhibited *in vitro* antifungal properties against *Candida albicans* – the causal microorganism of vaginal candidiasis, with negligible effect on *Lactobacillus*, naturally present in the vaginal microflora that are responsible for maintaining the acidic pH of the vaginal environment. A 3 day *in vivo* irritation study of the cross-linked chitosan–pectin polymeric films placed in the vaginal cavity of female rabbits

showed no signs of inflammation, erythema, or edema. A comparison of the film to the marketed fluconazole gel formulation – Flucos^R showed similar activity with reduced frequency of administration due to the enhanced mucoadhesive nature of the films.¹³⁰

Similarly, pectin-coated cationic liposomes encapsulating sertaconazole were developed by Abdellatif *et al.* for efficient localized treatment of vaginal candidiasis. The liposomes were composed of soya PC, cholesterol, and cationic surfactant, and coated with pectin, which imparted mucoadhesive properties, as evaluated by mucin test using porcine mucin and also prolonged the release rate of sertaconazole. The ability to prolong the release of any drug is an essential parameter for vaginal formulations as it reduces the frequency of administration/application, allowing better patient compliance. Thus, developed pectin coated liposomes showed a release rate of 58.24% $\pm$ 1.69 was superior to uncoated liposomes (89.07 $\pm$ 1.91) for sertaconazole post 8 h. The pectin-coated mucoadhesive liposomes were further formulated into a gel by incorporation into 1% HPMC for vaginal application. *Ex vivo* permeation study with sheep vaginal tissue displayed 1.75 times higher sertaconazole retention time in the vagina when treated with pectin–liposome loaded gels than only the sertaconazole-loaded control gel, along with reduced drug permeation (2.47-fold control gel) which is desirable to control the systemic exposure of the antifungal drug.

Furthermore, *in vivo* assessment of the anti-microbial activity of pectin-coated sertaconazole liposome gel in ovariectomized female Sprague-Dawley rats subjected to candidiasis showed significant inhibition of microbial infection as compared to the control gel. The reduction in the microbial burden can be attributed to the sustained release of sertaconazole and prolonged residence time in the vaginal cavity. The presence of cationic surfactant in the liposome can also induce microbial cell membrane decomposition. Local administration of mucoadhesive liposome gels showed a notable decrease in the inflammatory cytokines TNF- α and IL-23 and immunoglobulins IgM and IgG in serum compared to the control gel rat groups. Lastly, histopathology study of rat vaginal samples exhibited mild sub-epithelial inflammation with intact mucosa for the mucoadhesive liposome gel-treated groups, whereas intact mucosa with necrotic debris in the keratin layer and dilated blood vessels were observed for the control gel

treatment. Thus, pectin-coated liposomal gel formulation showed promising activity and can be used for local vaginal drug delivery.¹³¹

10.7 Clinical Progress

In order to augment performance and functionality, alterations in the functional characteristics of pristine pectin have been undertaken by several researchers *via* multiple approaches like derivatization, conjugation, carboxylation, methylation, and grafting, to list a few. One such notable example is modified citrus pectin (MCP); thermally or chemically modified groups of citrus fruit derived polysaccharides. In recent times, MCPs like GCS-100 and Pecta-Sol have established their efficacy in cancer therapy by inhibiting ligands such as type C lectins or cytokines and galectin-3 (Gal-3) function.^{132,133} The antineoplastic actions of MCP include suppression of tumor growth by apoptosis activation, halting the cell cycle, chemotherapy sensitization of tumor cells, immune function restoration, and reduction of angiogenesis and metastatic potential.

A clinical study within a group of 26 individuals bearing various solid tumors, MCPs in the hydrolyzed form at a 5 gram dosage with a frequency of three times a day, was administered orally. Post eight weeks (two cycles) of treatment, about 11 individuals (*i.e.* 42.3%) had depicted a reduction in disease stability, and six individuals (23%) had maintained a stable diseased status for about two years.¹³⁴ Because the included subjects had aggressive and advanced tumors, these outcomes should be weighted. In addition, the study aimed at assessing the clinical benefit response, quality of life, and tolerability. On a similar line of research, other citrus pectins encompassing higher Gal-3 affinity have also been investigated, and modifications in already existing pectins are being undertaken for increasing allied antineoplastic potential.

Researchers undertook a multicenter randomized clinical study to verify and examine the clinical effects of pectin-containing oligomeric formula (POF) that transforms into a gel on coming into contact with gastric acid. Around 201 subjects were included in the study and divided into two groups, *i.e.*, test group and control group receiving standard polymeric formula (SPF). The incidence of composite events was noted in 15 of 98

and 30 of 100 subjects from the POF and SPF groups. In contrast, only two subjects reported diarrhea in the POF group indicating greater efficiency with minimal adverse effects.¹³⁵

It has been reported in several studies that 30% of patients have a biochemical relapse in prostate cancer post local treatment, which makes optimal treatment elusive. Though androgen deprivation therapy is considered effective in reducing prostate-specific antigen (PSA) level, its long-term effects are unknown, and it also results in significant toxicities. Hence the evaluation of new non-toxic galectin-3 inhibitors could be a practical approach in overcoming biochemical relapse in prostate cancer. In a recent study, Keizman *et al.* have evaluated the dynamics and safety of pectasol-c modified citrus pectin (P-MCP) treatment in biochemically relapsed prostate cancer (BRPC) patients. Non-metastatic, non-castrate 53 patients were included in a phase 2 study of which 59% had a depicted decrease/stabilization of PSA levels, 70% had shown improvement of PSA doubling time with metastases in none of them on scanning at six months. The study outcomes have suggested potency and safety P-MCP treatment for BRPC.¹³⁶

Yan *et al.* reported that PectaSol-C, an MCP family member, exhibited tumor growth inhibition potential against prostate cancer cell lines. The mechanism of inhibition was the blockade of the MAPK cascade. The results revealed a halted proliferation and facilitated apoptosis induction on assessing the effectiveness in the prostate cancer cell lines.¹³⁷ Further studies have established synergism of PectaSol-C with doxorubicin and paclitaxel in prostate and ovarian cancer cell lines, respectively.^{138,139} In both the studies, reduced tumor size has been noted; details are briefly outlined in Table 10.2. Presently, a Phase III (NCT; NCT01681823) clinical trial is ongoing for testing whether oral PectaSol-C administration in men with relapsed prostate cancer can alter or improve prostate-specific antigen (PSA) kinetics or not. In other such studies, MCP-treated HUVEC cells have shown lost cellular organization and motility,¹⁴⁰ significantly decreased tumor size, angiogenesis, and a degree of metastases.¹⁴¹

Table 10.2 Brief outline of modified citrus pectins as galectin-3 inhibitors.

Inhibitor	Target	Effect	Ref.
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Modified citrus pectin (MCP)	Xenografts of colon and breast cancer; cell lines of prostate cancer; advance solid tumor-bearing patients	Inhibition of tumor growth, angiogenesis, and metastasis; Induction of apoptosis; cell cycle arrest; increased sensitivity to chemotherapy; rebalance the T-cells surveillance.	72, 134, 137, 140, 141
PectaSol-C (a MCP form)	Ovarian and prostate cancer cell lines	Induction of apoptosis; inhibition of proliferation; synergic effect with chemotherapy	72, 138

10.8 Summary

Pectin is a plant-derived anionic polysaccharide that FDA approves as a GRAS material. Pectin is mainly used in food industries as a gelling agent, emulsifier, thickening agent, and stabilizer; however, owing to its appealing biocompatible and biodegradable nature, pectin has received tremendous interest in therapeutic delivery applications. Naturally, pectin is present in various forms indicating the different degrees of esterification, % methylation, and structural features such as branching that impact its physicochemical properties, including degradation behavior and the ability to assemble into different drug delivery platforms. Pectin has been widely used to make therapeutic delivery vehicles like nanoparticles, microsphere, nanofibers, hydrogels, and bioadhesive films either by simple chemical modifications like oxidation or combining with other biomaterials. Pectin-based vehicles showed great promises for delivering small drugs, peptides, and genes in various applications *via* oral, systemic, nasal, topical, ocular, and vaginal routes. Several studies have shown that coating of pectin on liposomes and other nanoparticle formulations augments their stability by imparting more negative charges, which helps to improve their tissue penetration ability. Increased cross-linking and reinforcement of pectin chains with other materials like collagen fibers, hydroxyapatite, and bio-glass help to tune its mechanical properties, thus making it a suitable substrate for wound healing and tissue engineering applications. Also,

pectin can withstand gastric acidic pH without undergoing substantial degradation left to its widespread use in oral drug delivery application. Similarly, due to its excellent mucoadhesive nature, pectin-based vehicles opened new opportunities for transmucosal drug delivery.

10.9 Challenges and Future Perspectives

One of the main challenges in pectin-based vehicles is their increased water absorption capacity under physiological conditions that leads to significant drug loss due to leaching. Such premature drug release impedes applications of pectin vehicles for small molecular drugs and short peptides. Additionally, drug loss due to leaching seemingly becomes more prominent in systemic delivery and delivery of drugs to tissues involving repeated fluid flow such as the vaginal surface and urethra. Although cross-linking pectin with other materials like HPMC, chitosan, and acrylates can minimize such issues up to a more significant extent, such changes are still to be validated over various drugs. Similarly, due to limited functionalities in pendant chemical chains of pectin, this material offers limited scope for surface functionalization using ligands like peptide, antibodies, and aptamer for target delivery applications. Nevertheless, a range of chemical modification and derivatization can turn the pectin into a more reactive substrate; however, that makes the process cumbersome, ultimately affecting its translation potential. Apart from that, there is very limited understanding of how pectin-based carriers interact with immune cells like dendritic cells, macrophages, and neutrophils. Future studies should focus on understanding the dynamic molecular interactions of pectin-based carriers with immune cells. This knowledge will directly benefit research on rationalized immunomodulation in the context of treating complex medical conditions like chronic inflammation and infectious diseases.¹⁴² Also, as pectin is widely used in food industries for making edible confectionery items and milk products, in this context, future studies can focus on extrapolating these items for drug delivery purposes. Lastly, future studies should also focus on demonstrating more simple and translatable approaches for developing pectin-vehicles for drug delivery applications. Such efforts will help to minimize the gap between research and translation of such technologies.

List of Abbreviations

DHA	Dihydroartemisinin
FA	Folic acid
FDA	Food and Drug Administration
FPPDH	Folic acid–pectin–eight-arm polyethylene glycol– dihydroartemisinin/hydroxycamptothecin
GFP	Green fluorescence protein
GIT	Gastro intestinal tract
GRAS	Generally regarded as safe
HMP	High methoxyl pectin
IG	Intestinal gastric
LMP	Low methoxyl pectin
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NPs	Nanoparticles
PEG	Polyethylene glycol
SEM	Scanning electron microscope
TEM	Transmission Electron Microscopy
XRD	X-ray diffraction

Conflicts of interest

The authors declare no conflicts of interest.

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¹ Prof. Rinti Banerjee was deceased at the time of publication.

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Chapter 11

Pullulan in the Delivery of Therapeutics

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11.1 Introduction and Background

Recent advances in the investigation of drug delivery systems are focused on finding suitable strategies for safer administration of various drugs. Trans-mucosal transferring systems could be considered as the first-line option for the delivery systems of different drugs, including the protein-based components, as a group of compounds with well-known therapeutic potential and wide scaled applications (immunization, genetic, and drug therapy). This strategy, in turn, demonstrates the development in adequate delivery systems, as protein-based components are very sensible compounds, that easily undergo decomposition in physiological medium, mainly owing to enzymatic occurrences and pH. Polysaccharides were previously ascribed to be promisingly applicable in the development of drug delivery systems. Therefore, some polysaccharides were exploited successfully for this purpose;

in particular chitosan followed by pullulan paved the way, in addition to demonstrating great compatibility for protein carrying.¹⁻³

Pullulan as a polysaccharide is known to be neutral, water-soluble, and can be easily produced from fungal fermentation of starch by the action of *Aureobasidium pullulans*.⁴ Pullulan macromolecules basically consist of α -(1,6)-linked maltotriose as repeating units, that in turn consist of three glucose units bonded together by α -(1,4) glycosidic bonds.^{5,6} Owing to the unique and advantageous properties of pullulan in being odorless, tasteless, and biodegradable, pullulan is superiorly applied as ecofriendly wrapping compounds for food supplements⁷ (Figure 11.2). Pullulan was also reported to be used in the enzymatic production of maltotriose syrup.⁸⁻¹⁰ In addition to this, pullulan could be molded as biodegradable, highly water-soluble, edible, and oxygen impermeable films.¹¹ Moreover, pullulan can be applied as adhesives produced *via* the dispersion of pullulan ester or ether in aqueous medium with a good foam retention character (Figure 11.2). On the other hand, pullulan could be applied as an emulsifying and stabilizing agent for different food materials. In other instances it could also be applied as a lubricant, gelling agent, and binder.⁷ It could be applied in oral caring compounds.¹² Pullulan can additionally be used in the development of blood plasma substitutes. More recently, pullulan and pullulan derivative nanoparticles have been investigated to be superiorly applicable as anticancer reagents.¹³ Moreover, it has been investigated whether pullulan is applicable for Advanced Sustainable Body- and for Skin-Contact purposes.¹⁴ In addition, a recent study considered the development of pullulan-based carriers applicable in the controllable release of lipophilic ingredients.¹⁵

Several advantageous characteristics were reported for pullulan biopolymers such as its adhesive capability and its compatibility to producing fibers and thin bio-films, which make it highly applicable in drug-delivery applications¹⁶ (Figure 11.1). The high hydroxyl content for pullulan additionally leads to biopolymers with inherent physiological activities with chemical flexibilities. A biodegradable character is a mandatory property of any compound to be considered in the application of drug delivery, and pullulan needs to comply with this, as, once pullulan is administered, the intermolecular glycoside bonds are hydrolyzed and subsequently it will undergo metabolism and eventually result in glucose.¹⁷ The capability for adhesion to the cell surface could also be ascribed as an advantageous character within the subsequent applications,

enhancing the carriers' residence time and subsequently, the potentiality for absorption of the encaged protein. In this consideration, apart from the represented role of pullulan biopolymer on the adherence of *A. pullulans* to live surfaces, such like leaves,¹⁸ cell adherence to a pullulan-based surface was studied on a number of occasions.^{19–21} Pullulan contains nine hydroxyl groups per monomeric unit that could be substituted for the synthesis of different derivatives. Derivatives differ in accordance to the nature of the polarity of the reagents and solvents.¹² Different derivatives could be produced *via* copolymerization, etherification, oxidation, amide formation, esterification, chlorination, sulfonation, *etc.* This chapter mainly focuses on the application of pullulan and its derivatives in the delivery of therapeutics.

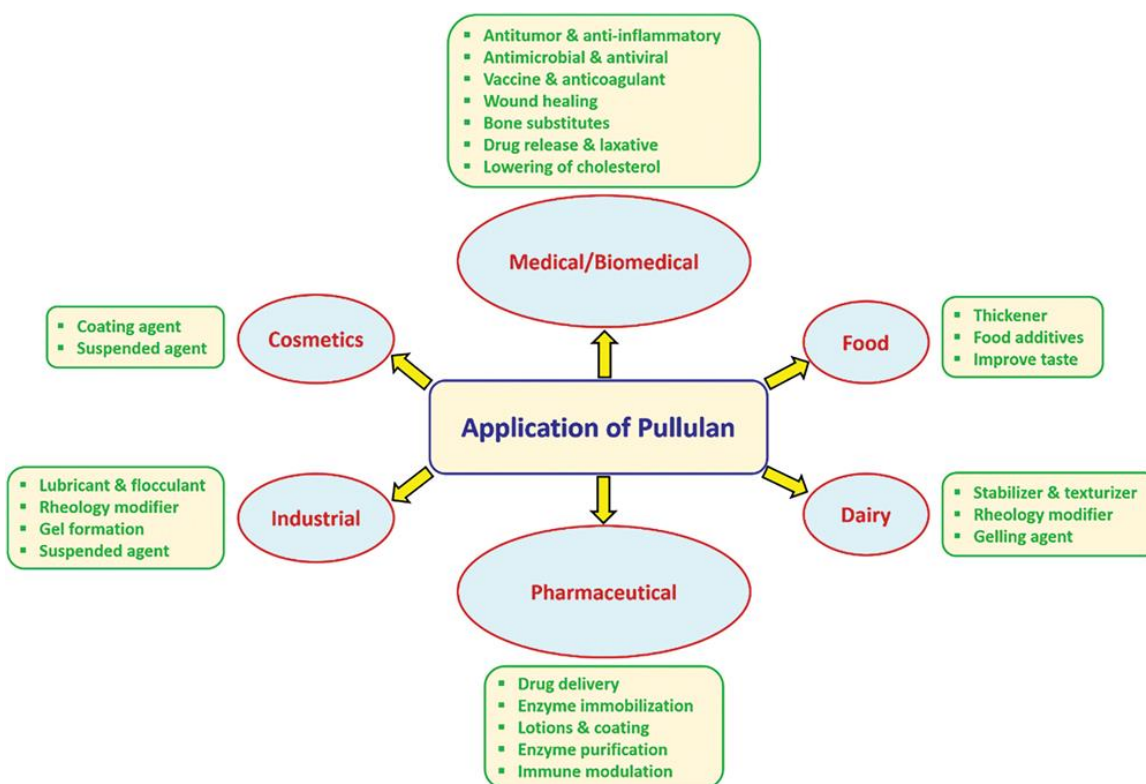


Figure 11.1 Schematic representing the different applications of pullulan/modified pullulan.

11.2 Pullulan as a Drug Carrier

Pullulan can be modified to be in different forms that have been reported to be superiorly applicable as drug carriers such as nano-gels, nanoparticles, or

microspheres. Pullulan in the form of nanoparticles could be inserted within cells *via* an integrin-mediated adhesive process.^{22,23} Integrin could be identified as the receptor for adhering to a cell to process the reaction between the nanoparticles and the cell. This type of interaction results in activation of the focal adhesion kinase enzyme that helps in the re-organization of the cytoskeleton,²⁴ resulting in endocytosis^{25,26} and subsequently in the successive insertion of the nanoparticles. Drug targeting could be categorized into passive and active targeting. Passive targeting could be proceeded *via* various routes of administration or toward epithelium without modifications of particle surfaces or after intravenous applications. Liver or spleen that are ascribed as the reticuloendothelial system could recognize the administered particle with lipophilic surfaces.²⁷ These kind of structures are supposed to be up taken with the macrophage through phagocytosis and allow selective accumulation of nano-carrier-based drug carriers in the required area.²⁸ In active targeting, the particle surfaces are modified with a targeting entity. The appropriate bio-specific recognizing mechanisms are followed by binding with the antibody or any other specific adhesive to the particle surfaces, while the surfaces of cells that could be recognized, are also modified with the counterpart like the antigen for a certain drug carrier to the action site that is a potential consequence of the intracellular uptake.^{29,30}

Pullulan is characterized with high affinities toward the liver owing to the presence of lectin-like receptors on the Kupffer and hepatocytes cells, that have high biological affinities for residues of sugar.^{31,32} These characteristic affinities for liver cells could lead to the accumulation of high molecular weight pullulan of nearly 80%.¹¹ Higher localization of pullulan within liver cells leads to shortening the half-life period in blood circulation. Another study has reported that pullulan could be complexed with the asialoglycoprotein receptor to undergo endocytosis and get inserted with higher affinities to the hepatocyte cell.³³ When the pullulan macromolecules are intravenously administrated along with arabinogalactan and asialofetuin, it leads to the reduction of the hepatic up-take of this polysaccharide. The hydroxyl groups orientation on the pyranose ring of pullulan acts in helping pullulan binding to the specific asialoglycoprotein receptor that further helps in endocytosis.³⁴ Interferon could help in controlling the activities of the disease of hepatitis C virus, however, if it was used at a high concentration, it could cause some side effects like psychoneurosis, leukopenia, and so on.³⁵ Pullulan–interferon

conjugation was reported to be exploited in liver targeting.³⁶ The interferon distribution through the whole body was ascribed to be proceeded *via* the interferon activation that is induced by oligoadenylate synthetase within the liver cells. Compared with the free interferon, the conjugate of pullulan is reported to require lower doses of interferon for liver targeting when intravenously injected. Cyclo-dextrin is essentially applied for enhancing the stability of drug, the bioavailability, and the dissolution rate.^{37–39} These could polymerize *via* crosslinking like using di-isocyanates or epichlorohydrin⁴⁰ or by binding to the pre-produced polymers. The microspheres of pullulan were supposed to be bonded with 1 : 1 cyclodextrin and epichlorohydrin (cross-linker). Any further diclofenac bonded with microspheres of cyclodextrin modified with pullulan was exploited as a drug symbol.⁴¹ Non-toxic and temperature dependant drug carrier templates composed of pullulan microspheres coordinated with semi-telechelic poly-(*N*-isopropylacrylamide-co-acrylamide) oligomer pendant were also investigated. It was reported that pullulan microspheres with long thermo-sensitive pendants with a concentration of 10 mg mL⁻¹ were shown to be a non-toxic template.⁴² The pendants give an off–on strict mechanistic reaction for drug delivering without any changes in the size of the polymer, considering the lowered critical temperature LCT.⁴³ Meanwhile, the pendants remain in the extended forms below LCST that restrict the drug release, however, above LCST they are collapsing to allow the drug delivering. The drug delivery *via* conjugation with pH sensitive pullulan macromolecules leads to a longer circulation time in blood, the retention of tumor cells becomes higher, and there is no hemolysis and lower cardiac toxicity than in unconjugated drugs.⁴⁴ Doxorubicin–pullulan pH sensitive conjugation is reported to be stabilized only at neutral pH.⁴⁵ The tumor cells differ in the composition and pH compared to the normal cells. When this conjugation comes in contact with cancer cells, the drugs are supposed to be released in tumor cells owing to the variation in pH. More recently, the affinity of pullulan produced *via* fungal fermentation of starch to be applicable as drug delivery templates was investigated.² More detailed study that was considered for designing pullulan-based solubilized micro-needled arrays in order to be applicable for enhancement of transdermal delivering of small and large biomolecules was also recently presented in order to approve the superiority of pullulan as drug delivery templates.⁴⁶

11.3 Pullulan Derivatives as Drug Carriers

Chemical modification of pullulan for producing various derivatives was performed *via* grafting with extended groups on the hydroxyl groups of each repeating unit, in order to improve its performance and its applicability (Figure 11.2). The functional groups of pullulan are specialized with their position on the glucoside units and their reactivities are also dependent on the reagent and solvent polarities. Pullulan copolymerization *via* grafting with butylacrylate was previously studied, while the pullulan macromolecular structure was modified by substitution of hydroxyl groups with L-lactide, acrylamide, methacrylate, or oxazoline to prepare *grafted* hydrogels.⁴⁷ These prepared hydrogels were endowed with interchangeable ionic, amphiphilic, chaperone-like, or thermo-responsive characters for developing drug carriers.⁴⁸ On the other hand, pullulan and its derivatives with short-chained alkyl groups (less than ten carbons) are capable of solubilizing the hydrophobic grains within hydrophobic compounds. Moreover, the derivatives with longer alkyl groups (up to ten carbon atoms) greatly increase the viscosity of colloidal solutions. The drugs could be uploaded on these derivatives in order to target certain organ cells.

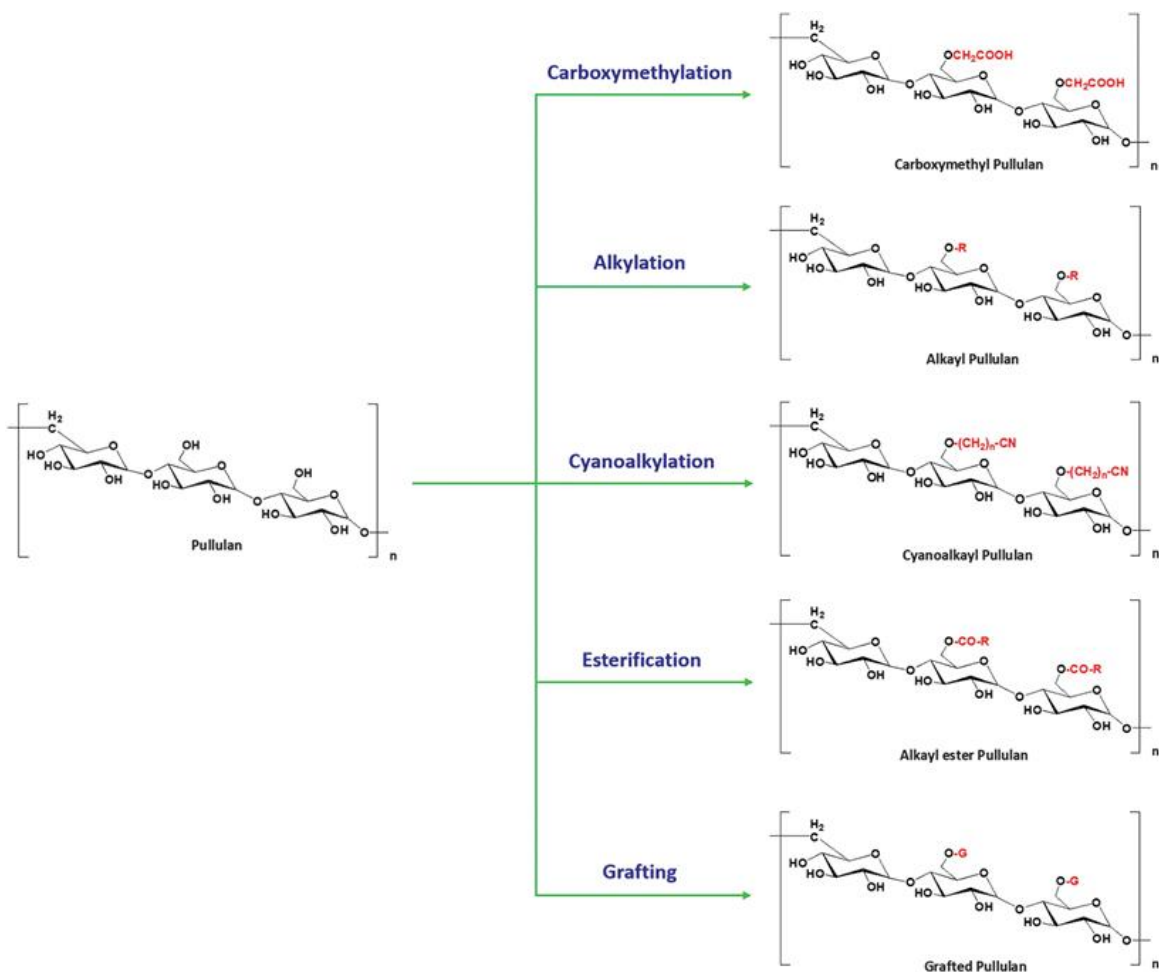


Figure 11.2 Chemical formula of modified pullulan.

Pullulan could be chemically modified to give one or more forms *via* carboxymethylation. Carboxymethyl pullulan (CMP) can be synthesized *via* the interaction of pullulan with isopropyl alcohol in the presence of sodium chloroacetate. Carboxylation of the pullulan backbone with carboxylate groups resulted in charging CMP with negative charges and CMP was sensitive to pH and ionic strength.⁴⁹ Negatively charging resulted in high M_{wt} ⁵⁰ and improvement of the vascular permeabilities.⁵¹ More recent, Li *et al.* (2014) reported a study of targeting fibroblasts, liver tumors, and cervical cancers by uploading of doxorubicin on CMP as a template at a pH of 4.50–7.40 (Table 11.1). It was investigated that nanoparticles of CMP–DOX acted in exhibition of pH responsivity and were characterized by higher targeting action of the liver in a pH of 4.50–6.50.⁵² Nogusa *et al.* investigated that CMP (>70

kDa) acted as a delivering template for drugs *via* peptide spacing structures and showed fewer systemic toxic effects than doxorubicin.⁵³ Characters of drugs, macromolecules, and intermolecular spacing structures between them affected the drug releasing to the target cells.^{54,55} *In vivo*, a series of conjugated CMP–doxorubicin with various intermolecular spacing structures, exhibiting different lengths and positions, were estimated for their anticancer effect. Various reports were considered showing that with peptide intermolecular spacers conjugates of CMP–doxorubicin bind through suitable dipeptide spacers and have more potential than doxorubicin. Masuda *et al.* reported that immune cells could target with conjugated immunosuppressing agents CMP for treating of various autoimmune diseases.⁵⁶ A novel immunosuppressing agent known as PA-48153C uploaded on CMP exhibited with suppressing effects on the adjuvant arthritis of the treated rats. Another derivative of pullulan containing two jeffamine units (Jeff M2005 and Jeff M600) was investigated with anionic thermosensitive characters, as it was synthesized through the interaction between CMP and jeffamine building blocks. The obtained derivatives were found with amphiphilic, polyelectrolyte, and thermosensitive characters. The thermosensitive property was owing to the existence of jeffamine that was poly-alkyleneamine (polyethylene oxide, *i.e.* PEO and polypropylene oxide, *i.e.* PPO). Owing to the previously-mentioned characters, such derivatives could be exploitable in potent purposes for controllable drug releasing.⁵⁷ More recent, Mocanu *et al.* prepared thermo-associative CMP nanoparticles, while periodate acted in oxidizing CMP (with accessible CHO groups) and was conjugated with two jeffamine molecules (ED 600 and ED 2003) in aqueous medium to be in turn hydrogenated under the effect of sodium borohydride.⁵⁸ Nanoparticles of jeffamine could retain some lipophilic (alpha-tocopherol), acidic (diclofenac), and alkaline (methylene blue) drugs. They additionally exhibited antioxidizing activities and approved the perspectivity for controllable drug delivery purposes. Additionally, they could exhibit potential applications in biomedicine for targeting the biologically reactive components owing to the metal retaining character (on amine functions). By comparing with the other organs, CMP showed a higher efficiency towards lymph nodes and the spleen.⁵⁹

Table 11.1 Summarized overview of modified pullulan as drug carriers.

Modified pullulan	Drugs	Drug amount (mg mg ⁻¹ pullulan)	Targeted tissues/organs	Ref.
Carboxymethyl pullulan	Doxorubicin	0.6–0.7/10.0	Rate liver	Nogusa <i>et al.</i> (1995) ⁵⁰
	Doxorubicin	0.6–0.7/10.0	Cancer cells	Nogusa <i>et al.</i> (2000) ⁵⁴
	Doxorubicin	0.7–1.7/10.0	Rate cancer cells	Nogusa <i>et al.</i> (2000) ⁵⁵
	Doxorubicin	0.3/10.0	Mouse breast cancer	Lu <i>et al.</i> (2010) ⁴⁴
Pullulan acetate	Indomethacin	40.0–80.0/40	Body joints	Na <i>et al.</i> (1997) ⁶⁷
	Clonazepam	10.0– 40.0/40.0	Brain	Young-II Jeong <i>et al.</i> (1999) ⁶²
	Adriamycin	10.0/50.0	Breast cancer cells	Na <i>et al.</i> (2003) ⁶⁶
	Adriamycin	20.0/50.0	Liver cancer cells	Na <i>et al.</i> (2003) ⁶⁶
	Doxorubicin	20.0/50.0	Cancer cells	Na <i>et al.</i> (2004) ⁶⁸
	Clonazepam	10.0/20.0	Brain	Jung <i>et al.</i> (2003) ⁶¹
	Epirubicin	5.0/50.0	Throat cancer cells	Zhang <i>et al.</i> (2009) ⁶⁴
	Silymarin	10.0/20– 10.0/40.0	Zebra fish embryo	Kumar <i>et al.</i> (2012) ⁶³

Pullulan succinylate	Lysozyme	100.0/1000.0	—	Fundueanu <i>et al.</i> (2008) ⁴³
	Diclofenac	150.0–200.0/10.0–50.0	Intestine	Constantin <i>et al.</i> (2011) ⁹⁶
Cholesterol@pullulan	Carbonic anhydrase B	0.05–1.0/1.0	—	Akiyoshi <i>et al.</i> (1999) ⁹⁰
	Fluorescein derivative	1.5/37.4	Human lungs and liver Rate liver and spleen Rate liver & spleen	Taniguchi <i>et al.</i> (1999) ⁹¹
	Insulin	2.0/10.0	Blood	Akiyoshi <i>et al.</i> (1998) ⁸¹
	Doxorubicin	30.0/100.0	Mouse fibroblast, human liver cancer and cervical carcinoma cells	Li <i>et al.</i> (2014) ⁵²
	Mitoxantrone	1.0/5–1.0/15	Spleen, heart, kidney, liver and lungs	Yang <i>et al.</i> (2014) ⁹²
	Doxorubicin	20.0/50.0	Cancer cells	Na <i>et al.</i> (2006) ⁹³
Deoxycholic acid@pullulan	Doxorubicin	10.0/50.0	A2780 cells	Lotan (1995) ⁹⁵
Retinoic acid@pullulan	Doxorubicin	2.0/50.0	Human cervical carcinoma cells	Lee <i>et al.</i> (2013) ⁹⁴

Folate-maleilated@pullulan	Doxorubicin	—	Cancer cells	Zhang <i>et al.</i> (2011) ⁹⁸
Poly(l-lactide@pullulan	Doxorubicin	2.0/40.0	HeLa cells	Seo <i>et al.</i> (2012) ¹⁰⁰
Poly(dl-lactide-co-glycolide)@pullulan	Adriamycin	10.0–20.0/40.0	Cancer cells	Jeong <i>et al.</i> (2006) ⁷⁰
	Doxorubicin	2.0–20.0/40.0	Cancer cells	Cho <i>et al.</i> (2009) ⁹⁹

Pullulan acetate (PA) is also another type of pullulan derivative that can be synthesized *via* treating of pullulan with acetic anhydride using formamide and pyridine. By self-agglomeration of PA in aqueous medium, a stabilized PA micelle with lipophilic cores and lipophobic outer covers was synthesized with a small size of nearly 100 nm and was subsequently exploitable in long circulation and target drug delivery to cancer cells.⁶⁰ Jeong *et al.* investigated that PA nanospheres could encapsulate lipophilic drugs (clonazepam, silymarin, *etc.*) in their cores and could in turn be applicable for treating some diseases such as panic disordering, cancer, *etc.*^{61–63} (Table 11.1). Moreover, Jung *et al.* (2003) investigated that more concentrated doses of drug could lead to a higher degree of acetylating reaction, resulting in a slower rate of drug release.⁶¹ Epirubicin is a well-known and highly applicable drug for the treatment of throat cancer, and when it was encaged within PA nanoparticles it showed higher cytotoxic effects for the tested tumor cells than the native epirubicin.⁶⁴ Further modification of PA nanoparticles with folate could be carried out, where the folate receptors could successfully act as the binding agents. FPA conjugated with epirubicin when compared with the original PA was ascribed to exhibit more affinity of drug entrapping, faster drug releasing, more cellular up-take and higher cytotoxic effects toward human nasopharyngeal tumor cells.⁶⁵ Additionally, PA nanoparticles for a pH sensitive drug target could be also applied for treating of some diseases such as ischemia, tumor, *etc.* under lower extracellular pH than the other cells and blood. pH sensitive systems could be treated with sulfadimethoxine or oligo-sulfadimethoxine to improve the affinity of drug delivery at a pH lower than 6.80.⁶⁶ Na, Jeong, and Lee (1997) studied the *in vitro* efficiency of PA nanoparticles, while PA was conjugated with anti-inflammatory

(indomethacin)⁶⁷ (Table 11.1). They investigated that ninety percentage of indomethacin was released at pH 7.40, while PA nanoparticles could additionally be loaded with sulfonamide for treating of MCF-7 as a type of breast cancer cells.^{66,68} More recently, Bishwambhar and Suneetha (2012) studied the effectiveness of pH on releasing naproxen *via* naproxen-conjugated pullulan acetate microspheres and decided that the drug release was seventy-five times higher at pH 7.20 compared with at pH 1.20. Biotinylated pullulan acetate (BPA) acted as a potent drug delivery template for treating of cancer.⁶⁹

Na, Lee, Park, *et al.* (2003) synthesized BPA by complex formation of PA with vitamin H for treating of cancer tumors⁶⁶ (Table 11.1). Vitamin H is well-known as a ligand for appropriate binding of nanoparticles and for efficient drug release for treatment of cancer cells. Adriamycin was conjugated with 3 types of BPA, by differing with the vitamin H groups per one hundred anhydro-glucose molecules of PA, as BPA nanoparticles were shown to exhibit certain interactions with HepG2 cells *via* the ligand–receptor binding and entering the cells *via* endocytic mechanistic action. Moreover, the degree of binding between nanoparticles and cancer cells increased with the increment of the contents of vitamin H, however, decrement in the efficiency of uploading and adriamycin content was due to lower hydrophobicity.⁷⁰ Jung, Jeong, Kim, and Kim (2004) synthesized another derivative (PEP) by modification of PA with carboxy-methylated poly-ethylene glycol, while, PA and CMPEG were prepared with two ratios to obtain PEP1 (forty two percent of CMPEG in PA) and PEP2 (nearly thirty nine percent of CMPEG in PA).⁶¹ CMPEG demonstration to PA led to a PEP *grafted* polymer exhibited with preferable characters such as avoiding up-take by macrophages, absorption of protein-based substances, and desirable bio-distribution. It was reported that PEP showed good compatibilities along with the capability to manipulate the releasing advantage of hydrophobic drugs.⁶¹ Park *et al.* (2007) prepared the ionic strength sensitive PA nanoparticles for delivering of radioisotopes (^{99m}Tc) targeted to the cancer cells, so that the normal cells were not deteriorated.⁷¹ PA nanoparticles were synthesized in an aqueous medium that is characterized with a zero ionic strength. These nanoparticles were uploaded with ^{99m}Tc and demonstrated to the body with ionic strengthening of 0.150. Nanoparticle agglomerates were attributed to their sensitivity toward ionic strengthening to be aggregated within cancer cells. They summarized that the rate of retention percentage of ^{99m}Tc in cancer cells is lower than ^{99m}Tc loaded

with PA nanoparticles. Therefore, radio-therapy can be expressed as an effective therapeutic technique for treating cancer cells.⁷¹

Succinylated pullulan (SP) as a type of pullulan was prepared by interaction of succinic anhydride and pullulan.⁷² SP could be further applicable as a drug delivery template for the preparation of polymeric drugs. To synthesize conjugates of polymer and drug, the interchanging of polysaccharide to be a reactive derivative is often needed. Such derivatives helped in suitable attaching and appropriate functionalities of drugs or bioactive components within polymeric chains.^{1,73} SP microspheres (SP-Ms) were reported to be stable towards acidic medium and swelled up to twenty times by reaching intestinal fluids.^{43,74} SP-Ms and poly-vinyl alcohol microspheres (PVA-Ms) conjugated with diclofenac were impregnated within CAB (cellulose acetate butyrate) shells for drug delivery to the intestinal system (Table 11.1). Pressure resulted in a high degree of swelling SP-Ms leading to rupture of CAB shells and diclofenac conjugated with PVA-Ms was liberated. SP-Ms as pH and temperature sensitive compounds were synthesized *via* grafting of pullulan with poly-(*N*-isopropyl acrylamide-co-acrylamide) before treating with succinic anhydride.⁴³

Cholesterol bearing pullulan (CHP) as one of pullulan derivatives could be synthesized *via* chemical interaction between the cholesterol group and the main backbone of pullulan with sodium mono-chloroacetate and alkylene diamine with 1-ethyl-3(3-dimethylamino)-propyl carbodiimide hydrochloride. CHP could be a modified lipophile and is ascribed as a superior template for hydrophobic substrates.^{75,76} In an aqueous medium, CHP simultaneously aggregates to compose hydrogel nanospheres that depend upon their concentration in reaction solution.⁷⁷⁻⁷⁹ The reaction mixture starts to seem like a viscous material at a concentration of 2%, while the polymer starts in self-aggregate at a concentration >3.5%.⁸⁰ Akiyoshi *et al.* in 1998 exploited a lipophile prepared CHP as a template for insulin carrying (Table 11.1). The authors reported that the CHP–insulin complex exhibited better stabilization and bioactivities than the pristine insulin.⁸¹ During 30 minutes of CHP–insulin complex up-take, insulin started to be complexed with blood, leading to 50–60% decrement in the level of glucose in the blood stream. Nanogels of CHP are applied in treating neurological disorder, and are specialized with the production of solubilized toxin aggregations, like in the case of Alzheimer's and in replacing antibody immunotherapy.⁸² The neutral nanogels of CHP

could interact with both monomers and oligomers for subsequent reduction in the toxicity for both the primary cortical and microglial cells in the central nervous system. Nanoparticles were reported to help in the purpose of drug delivery for targeting the neuron cells through the blood–brain barrier.⁸³ Complexes of hydrogel could be synthesized with hyaluronan gel and CHP.⁸⁴ Such hybrid hydrogels were found to help in the sustained release of protein-based drugs such as insulin, in addition to playing the role of chaperone *via* inhibition of any protein drug denaturation. Sustained release of drug from this type of hydrogel could decrease the drug costs, the frequency of injections, and the adverse effects of continuous injection. In addition to this, low drug dosage is efficient for drug targeting,⁸⁵ while high drug dosage pH-sensitive pullulan-conjugated nanostructures were used for hepatic targets.⁵²

The chemical modification of the building backbone of CHP with amino groups (CHPNH₂) is a newly investigated drug delivery template that could be exploited in the formulation of nanoparticles containing drugs (Table 11.1). Satoh *et al.* in 2008 approved the superior *in vivo* and *in vitro* efficiency of CHPNH₂ as a drug delivering template especially for an anticancer drug (docetaxel). In case of *in vivo*, the duration of activity for the drug complexed with modified CHP was longer, while the tumor cell ingrainings were diminished by comparing with the native drug by using the pleural dissemination mouse models. On the other hand, in the case of *in vitro*, the lung tumor cell lines were taken with a dosage of docetaxel–pullulan–NH₂ complexing template that showed a decrement of cytotoxic effects. Pullulan liposomes with nanogels and nanoparticles were also investigated to act as a good template for drug carrying. Sunamoto *et al.* studied the efficient preparation of the immune-liposomes with fragmented monoclonal antibodies as sensory devices and lipophile-modified CHP.⁸⁶ They found that the immune-liposomes interacted with certain amounts of sensory devices *via* non-covalent bonding so the antibodies retained their properties in order to be integral until reaching the sites of targeting.^{87–89} The thermal stability of CHP-based hydrogel nanoparticles was studied with carbonic anhydrase Brefolding.⁹⁰ Taniguchi and co-workers in 1999 also successfully synthesized galactoside carrying oil droplets or liposomes coated with CHP. So that it could be summarized that CHP liposomes could be helping in the site of drug delivering purpose for liver targeting both *in vitro* and *in vivo*.⁹¹ In more recent years, simultaneous-agglomerated biotin-modified cholesterol carrying pullulan was synthesized.⁹²

This novel approach led to efficient targeting of the lipophilic drugs for treating of cancer cells.

Self-organized nanogel through crosslinking of deoxycholic acid with pullulan was carried out in order to use it for drug delivery.⁹³ The synthesized nanogel of deoxycholic acid@pullulan was employed in the release of the anticancer drug doxorubicin. The release rate of the drug was inversely proportional to the substitution degree of deoxycholic acid onto pullulan.⁹³ More recently, Lee *et al.* (2013) synthesized all trans retinoic acid (ATRA) conjugated with nanogel pullulan (PURA). ATRA is investigated to help in the enhancement of the antitumor effects of the drugs.⁹⁴ ATRA was recognized as antitumor therapeutic reagents toward different tumors including skin, breast, kidney, neck, lung, *etc.*⁹⁵ (Table 11.1). Poly(α -lactide-co-glycolide) *grafted* pullulan (PulG) as one of the hydrophobic derivatives of pullulan was prepared by Jeong *et al.*⁷⁰ With an increment in the amount of the uploaded drug or the estimated M_{wt} of PulG, the drug releasing rate decreased. Such polymer complexing led to sustained drug release through dispersion methodology. PulG could be prepared by conjugation of pullulan building blocks with poly(α -lactide-co-glycolide) with the existence of DCC and DMAP at ambient conditions. Poly((3-acryl amide propyl)-trimethylammoniumchloride)-*grafted* pullulan is known to be essential for exhibiting the polymer with wide-scale medical applicability, especially in drug delivery purposes.⁹⁶

Lu *et al.*, also studied the preparation of folate-conjugated pullulan that could be synthesized by treating folic acid with pullulan under ambient conditions with the existence of DCC (1,3-dicyclohexyl carbodiimide) and DMAP (4-dimethyl amino pyridine), while folate conjugated pullulan could easily act in targeting the tumor cells in different organs such as brain, kidneys, ovary, lungs, or breast, that are well known to be the most difficult types of tumor cells to be treated.⁹⁷ However, folate receptors are characterized with very limited accessibility for the healthy cells than the tumor cells, owing to their position on the polarized epithelia's apical membrane. Exploitation of these receptors resulted in increment in the therapeutic index of the drugs, as by increment in the density of the cancer cells, the receptors densities were found to also be increased. Zhang *et al.* (2011) investigated a unique folate conjugated maleilated pullulan (FAMP) doxorubicin.⁹⁸ While FAMP was prepared by pullulan treated with maleic anhydride under the effect of nitrogen gas. FAMP was consequently reacted with folic acid to make this type of

conjugated component pH sensitive. Application of this type of conjugate improved the cellular up-take of the drug and increased the cytotoxic effects (Table 11.1). Conjugates with pH sensitivity are known to result in more efficient drug delivery inside acidic tumor cells.

Cho, Park, and Na previously mentioned in their research report that pullulan-*g*-poly-L-lactide could help in controllable drug release^{99,100} (Table 11.1). Pullulan could be conjugated with PUPL by acylating with acetic anhydride and L-lactide in the existence of TEA (triethylamine) at 70–75 °C. Thermo-sensitive PUPL nanogels helped in the degradation of cancer cells *via* controllable and long-term drug delivery action. Wang, Chen, Yang, Yang, and Liu prepared nanogels with reversible light-response to be investigated as a highly applicable drug delivery template. The authors studied the preparation of conjugated pullulan with carboxylic containing spiro-pyrane (Sp) compounds. These Sps were colorless, light-tunable, spiro form compounds which only absorbed ultraviolet irradiation. Moreover, the isomerization of Sp under ultraviolet irradiation or thermal energy, it interchanged to merocyanine (Mer) compound, that could be changed back to Sp form under exposure to ultraviolet irradiation. Spiro-pyrane conjugated with pullulan (SpPs) could be self-assembled into nanogels in aqueous media, that could be characterized and exhibited a reversible sensitivity to irradiation. Pyrene uploaded nano-gels were shown to exhibit light controllable releasing of the drug. SpP is a new stimulus-sensitive amphiphilic nanogel which could be significantly effective in drug delivery purposes and tissue culture.¹⁰¹

Bruneel and Schacht synthesized an amine-containing pullulan by activation with chloroformate. The authors mentioned that, for the appropriate conjugation of polymer and drug, polymer derivatization is needed.⁷² The pH-sensitive or thermo-sensitive blocks or both of them could be conjugated with pullulan polymeric chains *via* addition of amine group reactions.¹⁰² These blocks could be conjugated with pullulan by chemical bonds of pullulan aldehyde groups and amino groups of the blocks. Principally poly-ether-amine or poly-(ethyl oxide)–poly(propyl oxide) amine was applied. This type of conjugated structures could help in easier targeting of tumor cells and controllable drug releasing. For achieving efficient pH sensitivity, carboxylic acid groups or amino groups could be added that exhibited specific pK_a values. For a thermosensitive character, pullulan could be conjugated with organic polymers such as a poly-*N*-isopropyl acryl amide or poly-ethylene oxide–poly-

propylene oxide that was characterized to be thermally sensitive.^{103,104} Most recently, lipophilic *grafted* pullulan nanocarriers were prepared and investigated for percutaneous delivering.¹⁰⁵ Yuan *et al.*, also studied the preparation of the nanoparticles of cholesteryl-modified aminated pullulan for evaluation of lipophilic degree on drug releasing and cytotoxic effects.¹⁰⁶ Moreover, Mehdi *et al.* also reported a study about fabricating nanofibers designed from rizatriptan-loaded pullulan to be subsequently applicable as an oral fast-dissolving drug system.¹⁰⁷

11.4 Pullulan-based Therapeutic Laborers with Bactericidal and Fungicidal Activities

Interaction between any substance and the pathogenic reagents from surrounding environments such as algae, bacteria, viruses, or fungi mainly depends on various key factors such as the concentration of substances, chemistry of cell surface, the presence of charge on the surface, temperature, pH, and culture age. Considering the heterogenic nature of the outer layer of the bacterial cells, complexing of biological molecules like lipopolysaccharides, peptidoglycan, phospholipids, teichoic and lipo-teichoic acids that possess various functional groups such as amine, phosphoryl, or carboxyl groups positioned in different associative sites that ensure the interaction with the antibacterial reagents *via* hydrophobic associations like dipole–dipole interactions, ionic or covalent bonding, and steric interactions. Except for the existence of different protein-based components within the inter-composition of the bacterial cell wall, for example, there are significant morphological differences between Gram positive and Gram negative cells.¹⁰⁸

In accordance with the various reports considered in studying the fungal cell wall, it was approved that this type of cell wall is primarily constructed of glycoproteins, mannans, glucans, and chitin covalently bonded together.¹⁰⁹ *Ciprofloxacin*, an antibiotic reagent that is often applied in the treatment of bacterial keratitis was reported with enhanced water solubility when it was impregnated with a polymeric amphiphilic system based on *N*-octyl-*O*-glycol chitosan¹¹⁰ or poly-(*dl*-lactide-*co*-glycolide)-*graft*-pullulan.⁷⁰

A Garhwal *et al.* study was considered with the encapsulation of the lipophilic free basic form of medicine with core–shell nano-spherical structures based on a pullulan–poly-caprolactone (PULL–PCL)-block

copolymer. The produced nano-spherical structures with a diameter of 142 nm were exploited in preparation of a therapeutic contact lens manufactured from poly-hydroxyl-ethyl-methacrylate (pHEMA) and poly-ethylene glycol dimethacrylate (PEGDM) as the UV-cross-linking agent. Moreover, the reproduction of culture incubated within 10^8 or 10^7 bacteria per mL of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively, was effectively retarded in growth by lower than $2 \mu\text{g mL}^{-1}$ of nano-spherical ciprofloxacin conjugated solutions.¹¹¹

Di Meo *et al.* investigated a novel nano-hydrogel template for testing the uploading and releasing of *Levofloxacin antibiotic reagent* with the aid of hydrophilic molecules. Amphiphilic hydrogels resulted from the esterification reactions of pullulan-4-(di-methyl-amino)-pyridine using a bromo-hexyl derivative of riboflavin tetra-butyrate and di-methylsulfoxide. Consequently, cyto-compatible polymeric colloids with a particle size of 210 nm, zeta potential in water of -18 mV , and poly-dispersity index of 0.2 were estimated via autoclaving sequentially to the sonication of macromolecules in the reaction solution.¹¹² New investigative hydrogels consisting of *Clotrimazole*-coated nano-capsules of Eudragit[®] RS100 were developed with impregnation of pullulan and poly-(acrylic acid) named Pemulen[®] TR1 for medical treating of vulvovaginal candidiasis. One of the semi-solid formulations *in vitro* controllably released the drug-coated nano-capsules from the prepared hydrogels ($20.14 \mu\text{g cm}^{-2}$ within 8 hours). Owing to the hydrogels containing nano-encapsulated clotrimazole exhibiting low penetration efficiency for cow vaginal mucosa ($14.00 \mu\text{g cm}^{-2}$), the drug molecules were stabilized within the mucosa surfaces, while drug absorption was diminished.¹¹³ Pullulan nano-based nail formulations for treating of onychomycosis were created by mixing the dispersion of *Tioconazole*-loaded lipid nano-capsules coated with cationic chitosan with pullulan solution. Novel pullulan nano-based nail formulations were ascribed to be a lower irritant reagent rather than commercial formulation, owing to the nano-encapsulated systems promoting the impregnation of smaller quantities of drug molecules, but with comparable therapeutic actions.¹¹⁴

To overcome the side effects motivated by the effect of *Terbinafine* in topical administrations for onychomycosis, this kind of drug could be enclosed within the liposome-coated pullulan membranes. The optimum membrane formulations for the antifungal activities were selected based on the drug

releasing profile (nearly 72%).¹¹⁵ Krull *et al.* successfully synthesized a pullulan-based stripped film through drying–casting of the wet-milled drug colloids in order to dissolve *Griseofulvin* as a poorly water-soluble drug, as an oral drug exploited in the treatment of fungal infections. The membrane characters could be formulated *via* controlling the concentrations of sodium dodecyl sulfate (surfactant) and xanthan gum (thickening agent), and drug uploading such that most of the membranes have rapid or immediate drug release (more than eighty percent for membranes solubilized within 30 minutes).¹¹⁶

11.5 Pullulan-based Anticancer Laborers

In accordance with Ringsdorf modeling, polymeric drug carriers consist of definite numbers of drug macromolecules, associated with polymeric building blocks *via* a *spacer*, while, spacer molecules incorporate pre-determined breaking points that ensure the drug release after cellular up-take of the conjugate molecules. The system could also have the *targeting moiety*, such as tumor-specific antibody, antibody fragmentations, or saccharides.¹¹⁷

Chemical modifications in polysaccharides for the production of characteristic derivatives with enhanced pharmaceutical activities. Attributing to increment of needing anticancer drug carriers in the previous decade until now, various biodegradable, biocompatible, and non-toxic carriers based on pullulan derivatives were prepared and examined for uploading and releasing of epirubicin, 10-hydroxycamptothecin, doxorubicin, mitoxantrone, or paclitaxel. Pullulan-based carriers for anticancer drugs include pullulan nanoparticles sensitive to pH, bio-conjugates, or self-assembling hydrophobic pullulan. In some cases, designing of the bio-conjugates is supposed to use a hydrazine bond sensitive to acids, stabilized at physiological pH, but it could be easily hydrolyzed under acidic conditions, for conjugation of the drug to the pullulan building blocks.¹¹⁸

Additionally, a lot of efforts have been invested for finding suitable ligands for the selective targeting of drug carriers to cancer tissues. Moreover, other key factors such as the pH of the media or hydrophobic–hydrophilic balancing, micro-particles, nanoparticles size, micelles, hydrogels, or conjugates uploaded with the drugs, detected *via* the laser light scattering technique, estimated the performance of anti-neoplastic drug carriers based on some derivatives of pullulan, specifically the drug contents, superiority of entrapping, and the drug

uploading efficiencies. Typically, the investigative antineoplastic drug carriers based on pullulan derivatives were estimated with dimensions in the range of 50–300 nm. In accordance with the Bae and Park approach, this size criterion is supposed to ensure the stabilization of such drug carriers for a long time in the circulatory system so that it could fulfill their therapeutic action. In agreement with certain medical cases, the doctors are appealing for specific drugs in the therapeutic scheme in order to accomplish good results: combinatorial or co-delivery therapeutic drugs. In co-delivery systems two drugs are uploaded within the same polymeric template and released in a controllable manner. Consequently, in the case of combining targeted therapies *versus* cancer cells, two types of drugs impregnated within distinct delivery systems were simultaneously supplied in the treating process; these acted in controlling their antitumor synergistic effect; by acting as a preventer for the invasion scheme for cancer cells and on the other hand by being anti-proliferative reagents. The drug carriers were the template of para-cellular pathways *via* tight junction or transcellular pathways by receptor-mediated endocytosis, and eventually reach systematic circulations *via* the endothelium, at the cellular level. While at macroscopic levels, the growing of remission or inhibition of tumor under treatment will be monitored.¹¹⁹ Recently, a unique delivery template for Mitoxantrone with nanoparticles of lipophilic-modified pullulan was investigated to be sequentially applicable in the inhibition of bladder cancer cells.¹²⁰

11.6 Pullulan-based Antioxidant Laborers with Radical Leaching Potentiality

Oxidative stress resulting in the generation of excessive amounts of reactive oxygen species (ROS) to be liberated in the biological systems under ordinary physiological conditions *via* a regulation pathway, can deteriorate DNA, cellular proteins, and lipids, resulting in the fatal lesions in cells that is accompanied with carcinogenesis.¹²¹ Overcoming the carcinogenesis and aging of the cell, it is vital for maintaining a balanced feeding with substances having anti-oxidative activities and free radical scavenging characters such as curcumin or rutin. These kinds of compounds need to be protected by variations in pH till they are eliminated from the circulatory system. Curcumin and rutin are well known as polyphenolic substances having anti-oxidative and

anti-inflammatory properties. Combination therapies that include the two substances were examined to enhance inflammatory bowel disease. Rutin, also known as rutoside, quercetin-3-O-rutinoside or sophorin, is a glycoside substance of flavonoid quercetin, composed of OH groups, and could be extracted from different plants, such as apple, citrus fruits, and capers, and could be exploited as anti-inflammatory, anticoagulant, or antioxidant agents. This kind of bio-flavonoid substance could be applied in fabricating some electro-spun anti-oxidative pullulan nano-fibers containing 4% w/v Pluronic F127 solid suspension and rutin.¹²²

By comparing with the pristine rutin, it looks that the uploading of drug on the smooth and porous nano-fiber represented an improved solubility and stabilization under ultraviolet irradiation, with concurrent retaining of its antioxidant activity, to exhibit a fast drug releasing profile. On the other hand, curcumin as the active component of turmeric is known as an ingredient which was recently investigated with antimicrobial, antioxidant, anti-inflammatory, cholesterol-decreasing, and anticancer actions, that superiorly could be adsorbed in organisms with the existence of Bioperine, the active component obtained from *Piper nigrum* plants. To enhance its therapeutic action, the researchers designed new pullulan-based carriers such as nanoparticles, micelles, or nano-gels for prolonging the stabilization of curcumin within the circulatory system or to enhance its toxic action toward carcinoma cells. Nanoparticles of Pullulan acetate uploaded within curcumin were proposed for treatment of hepatic diseases.¹²³

Curcumin-uploaded pullulan acetate nanostructures enhanced the impregnation efficiency, stabilization, and the therapeutic superiority of curcumin with sustained release under physiological conditions, and therefore, it countered the poor bio-availabilities rendered by their physiochemical property and poor stability and low solubility in aqueous media. Such novel nanoparticles were investigated as an efficient liver protective reagent *versus* the diethyl nitrosamine, which is a material extracted from tobacco that induces liver cell deterioration. Sarika *et al.* reported a study for the preparation of pullulan–curcumin conjugates without and with a targeting chelating agent for analyzing and comparing their cyto-toxicities differences in HepG2 cells. The referred approach was proceeded in a few steps as follows; pullulan was oxidized to give the pullulan aldehyde; then chemical modification of lacto-bionic acid with ethylenediamine took place to introduce the amino group that was needed for Schiff's base reaction with pullulan

aldehyde; in order to prepare pullulan aldehyde conjugates. Afterward the chemical modification of curcumin was proceeded *via* the addition of succinic anhydride to in-turn prepare pullulan–curcumin succinic anhydride conjugates and pullulan aldehyde curcumin succinic anhydride. In this approach, galactose containing lacto-bionic acid is chosen as the target chelating agent. Galactose-terminal molecules or conjugates are selectively known by asialoglyco-protein receptors represented on the sinusoidal surface of the hepatocyte which transport them to the lysosome within liver cells. Self-assembled micelles of pullulan–curcumin succinic anhydride conjugate with amphiphilic characters were identified to exhibit unimodal distributions with a hydrodynamic dimension in a range of 363 nm. This estimated value was determined by dynamic light scattering (DLS) detections and slightly differed from those estimated from scanning electron microscopic images (320 nm). The negative zeta potential of pullulan–curcumin succinic anhydride in pullulan aldehyde–curcumin succinic anhydride conjugates is due to the existence of non-reacted succinic acid molecules on the conjugate molecules, and affirmed the non-processing of the crosslinking reactions between curcumin succinic anhydride and pullulan. An *in vitro* drug releasing study carried out for two days within two different pH values declared a fast release of curcumin in acidic medium and a frequently slower release at neutral pH. Pullulan aldehyde–curcumin succinic anhydride conjugates exhibited selective and better toxicity toward liver cells while it manifested the targeting superiority of galactose moieties. Studying the cellular up-take for pullulan–curcumin succinic anhydride conjugates approved the ameliorated agglomeration of the galactosylated conjugates in liver cells through asialoglycoprotein receptor-mediated pathways. All the referred results declared that both conjugate micelles are appropriate candidates for carrying curcumin toward cancer cells in the liver. Polymeric micelles, nanoparticles, liposomes, lipid-based hydrogels, and nanoparticles were presented in solution for uploading low molecular weighted and hydrophobic polyphenolic compounds such as curcumin. These chemo-inhibitive and therapeutic reagents are widely exploited for liver pathologies owing to their potential antioxidant and anti-inflammatory activities.¹²⁴

For inhibition of its rapid systematic eliminations, poor aqueous solubility, unbalanced tissue absorptions, and decomposition in alkaline medium, Yuan *et al.*, prepared new delivery systems for curcumin based on glycyrrhetic acid–pullulan differing in the degree of substitution of glycyrrhetic acid, a vital

bioactive ingredient in traditional Chinese medicine liquorice with antiviral, anti-inflammatory, microbicide, antioxidant, and antitumor properties and immunomodulatory, liver protective, and cardio-protective characters. Self-clustered nano-sphere particles were synthesized *via* the dialysis methodology and were examined for their capacities to upload curcumin motivated by the physiological conditions; therefore, liquid chromatography estimated values of 10%, 7%, and 4% for the same weight per weight ratio of 10 for the drug/delivery template, relying on the substitution degrees of each type of nanoparticles. The release of curcumin was sustained and depended on the pH values. MTT studies reflected that curcumin–glycyrrhetic acid–pullulan nanostructures showed higher cytotoxic effects in liver cells than free curcumin, however, glycyrrhetic acid–pullulan nanoparticles exhibited non-significant cytotoxic effects. Moreover, curcumin–glycyrrhetic acid–pullulan nanoparticles can significantly enhance the stability, water solubility, and cytotoxic effects of curcumin in liver cells *in vitro*, which might be due to the liver targeting of glycyrrhetic acid and the inherent affinities of pullulan polymeric macromolecules for the hepatic cells.¹²⁵

11.7 Pullulan-based Therapeutic Laborers with Anti-inflammatory and Immunomodulatory Performance

Drugs of anti-inflammatory activities such as diclofenac, piroxicam, indomethacin, naproxen, and etanercept, and immunomodulatory medicine such as cucurbitacin B and silymarin could be successfully entrapped or encapsulated within pullulan, for the preparation of drug delivery systems (Figure 11.3). Besides their antipyretic and analgesic activities, *Indomethacin* is known as a nonsteroidal anti-inflammatory reagent with anti-inflammatory activities that forbid the activities of cyclooxygenase, and subsequently the production of prostaglandins related to inflammation, fever, and pain. Indomethacin was applied as one of the hydrophobic modeling drugs in order to be impregnated *via* different methodologies such as nano-precipitation and dialysis under ambient conditions, using organic solvents such as dimethyl sulfoxide (DMSO) or *N,N*-dimethyl formamide (DMF), into thermo-responsive pullulan-*grafted*-poly(*N*-isopropylacrylamide) (P-*g*-pNIPAM) copolymers. Higher uploading and entrapping efficiencies were given by

dropping in aqueous *N,N*-dimethylformamide solution containing both the drug and the copolymer. For an equal molar ratio of indomethacin/polymer, while the polymer concentration was 10 g L^{-1} , the drug entrapping efficiencies were up to 80 percent. The formation of indomethacin-uploaded pullulan-*g*-poly(*N*-isopropyl acrylamide) nano-distribution was due to hydrogen bonding between pullulan and indomethacin–poly(*N*-isopropylacrylamide). *In vitro* release of indomethacin from indomethacin uploaded pullulan-*g*-poly(*N*-isopropyl acrylamide) nanostructures at pH = 7.4 and 5, in phosphate buffer saline solution and acetate buffer, respectively, depended upon the M_{wt} of the poly-*N*-isopropylacrylamide building blocks composing the copolymers, and the temperature and indomethacin contents.¹²⁶

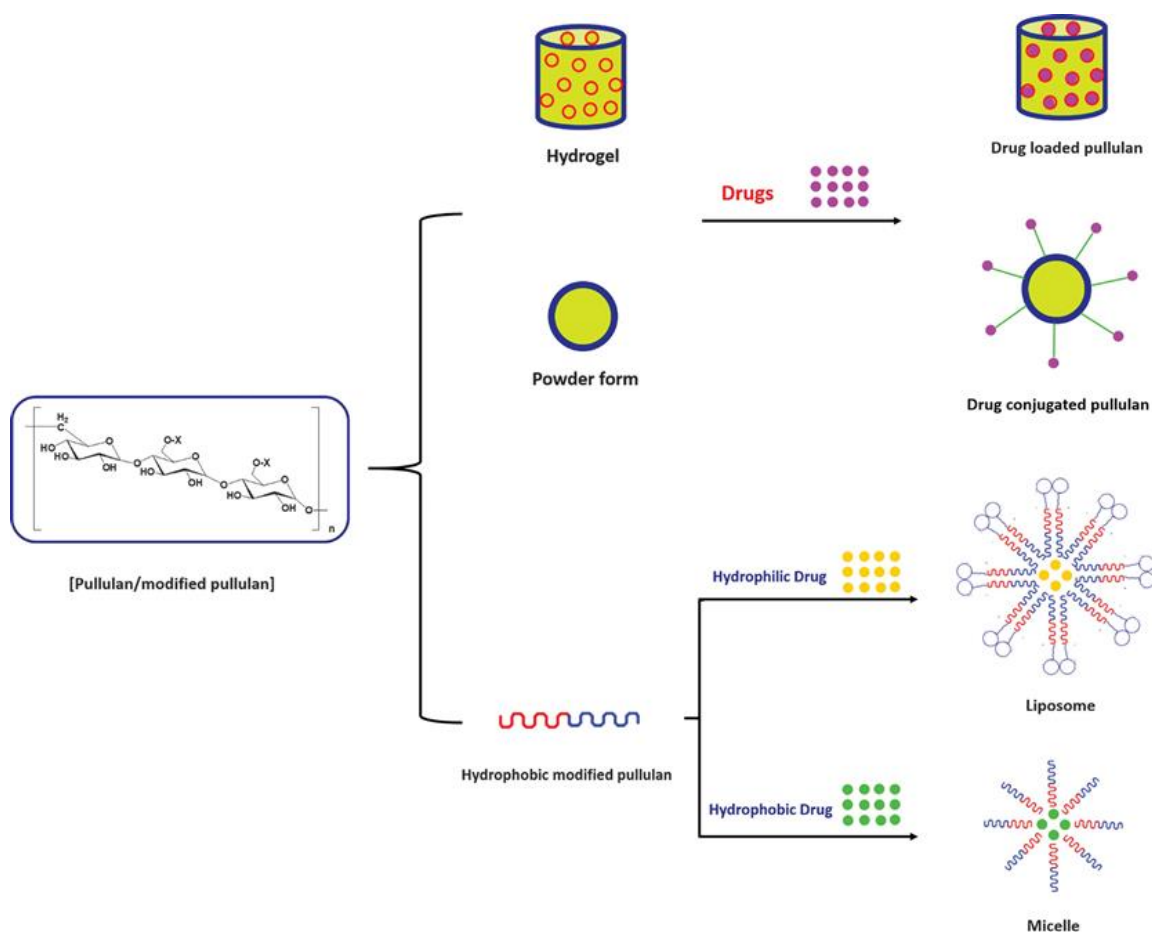


Figure 11.3 Different formulations of pullulan/modified pullulan in drug delivery.

Diclofenac or [2-(2,6-dichloroanilino)phenyl]acetic acid is well-known as the sodium salt of acetic acid nonsteroidal anti-inflammatory medical reagents, exhibiting similar mechanistic effects and actions to indomethacin. It is often exploited for the treatment of different symptoms of spondylitis and arthritis. Coating of the drug pellets with triacetate pullulan thin films by membrane-mediated pulsed laser vaporization methodologies, when retardation and controllable release of drug is required.¹²⁷ In this methodology, the irradiation produced by pulsed excimer laser sources, that are operating with repetitive rates was applied for ice targeting evaporation methods. Other researchers¹²⁸ synthesized some membranes from tertiary mixtures of hypromellose, polyvinylpyrrolidone, and pullulan as matrices for uploading sodium diclofenac. The authors considered application of the real-time spectroscopic analysis in conjunction with statistical design for optimizing and developing non-conventional intraoral carrying templates such as fast dissolving membranes. Considering these points, the authors compared the mechanical characters of blank films exhibiting a tensile strength of 2–49 MPa and for Young's modulus 1–21 MPa%, over 21–105 s as the time intervals, with that of diclofenac-uploaded membranes that exhibited with a Young's modulus and tensile strength for the film of 6–78 MPa% and 11.21 MPa, respectively. Additionally, the dissolution of the prepared membrane was shown to commence mainly the immediate drug release with 50% within 1 minute.

Mocanu *et al.* investigated the preparation of the anionic pullulan nanostructures for diclofenac carrying purposes. Thermo-associative nanostructures were produced *via* a cross-linking interaction between carboxymethyl pullulan (oxidized by the action of periodate) and two di-functional Jeffamines (ED-600 and ED-2003). The applied nanostructures exhibited spherical geometrical shaping, amphiphilic properties, and size distribution of 200 nm that could be ascribed as appropriate characters for diclofenac entrapping. Diclofenac release occurs gradually, while the less efficient cross-linked nanostructures release the medicine faster than the more efficient cross-linked one. During the first half hour, 18–32% of diclofenac with fast release is observed owing to the “burst effects,” and subsequently slower controlled and sustained release is detected within the incubation time, while, releasing amounts are estimated to be 72–99.5%. Under the effect of temperature of 40 °C, diclofenac was observed to be released much faster, with 70% during one hour.⁵⁸

11.8 Pullulan-based Therapeutic Laborers for Bone Illness

Pamidronic acid (named as 3-amino-1-hydroxy-1-phosphonopropyl-phosphonic acid *Pamidronate*) and Actonel (named [1-hydroxy-1-phosphono-2-(pyridin-3-yl)ethyl] phosphonic acid or *Risedronate*) belong to a type of drugs known as bisphosphonates which are mainly adsorbed within the bones to the calcium phosphate or hydroxyapatite crystals and might directly block the dissolution of these mineral ingredients of bones. These bisphosphonates are reported to be applicable in treating the Paget's diseases of bones, in order to inhibit osteoporosis, for reduction of the high calcium level in the blood stream related with malignancy, and to decrease the deterioration in bones resulting from the multiple myeloma or metastases of breast cancer. The skin, nerve tissues, cartilage, and bones can be reproduced and repaired by applying the cells and biomaterials for the drug-carrying templates for growing key factors and cell scaffolds. Bone repairing and healing are mostly followed up *via* magnetic resonance imaging, computer tomography (CT), and X-ray spectroscopy. Additionally, bone-specific multimodal imaging as a new technical analysis was started to be applied for visualizing the tissue reproduction of bones.²

Regarding the high efficiency of the pamidronate towards hydroxyapatite for bone tissues, Liu *et al.* (2012) synthesized pamidronate pullulan conjugates that contained two imaging probes, the first was Cy5.5 mono-functional *N*-hydroxy-succinimidyl ester Cy5.5/DTPA as a photoluminescent moiety and gado-pentetatedimeglumine Gd^{3+} -DTPA as the MRI reagent.¹²⁹ Colloidal solutions of the prepared conjugates, ascribed as pamidronate–pullulan–F/M referred to as Gd^{3+} -coordinated pamidronate–pullulan–Cy5.5/DTPA, were intravenously taken as doses at various time intervals for one month by mouse modeling of ectopic bone formation, produced *via* subcutaneous implantation with gelatin hydrogels impregnating bone morphogenetic proteins (BMP-2). It was expected that the histological examinations affirmed the pamidronate was well functioned for enhancement of the hydroxyapatite efficiency for pullulan and the *in vivo* agglomeration at the newly produced bone tissue. Therefore, this drug carrier template is approved to be non-invasive multi-modeling images for monitoring of bone reproduction, too. Some researching approaches were interested in the investigation of the polymeric micro-particles based on

pullulan and Eudragit[®] S100 by spraying–drying technical methodology¹³⁰ in order to facilitate the esophageal passages and decreasing the mucosal adhesion that could lead to gastrointestinal injury. Tablet ingredients could be prepared *via* the direct compression of risedronate-uploaded micro-particles through the direct tableting excipient such as microcrystalline cellulose, colloidal solution of SiO₂, stearate magnesium, and poly-vinylpyrrolidone. It was supposed that risedronate-based tablets synthesized within this pathway to decrease the possibilities for the local accumulation of drug closer to the gastrointestinal mucosa.

11.9 Conclusion

In recent years, numerous research studies have been extensively considered with the innovation of drug carrying templates that are challenged for fabrication from non-toxic, biodegradable, and biocompatible materials such as polysaccharides. Pullulan is one of the polysaccharides that is mainly composed of maltotriose repeating units. Pullulan could be produced *via* the fungal fermentation of starch under the effect of *Aureobasidium pullulans*. Pullulan and its derivatives could be coordinated in the form of a complex with special types of hydrophobic drugs. Therefore, the presented chapter reviews the superiority of pullulan in the delivery of various therapeutic reagents. Different research groups were interested in investigating the hydrophilic–hydrophobic balance and physical interactions in order to design different advantageous drug carriers with controllable uploading and selectivity for carrying the drugs to certain organs. Pullulan chemical construction could be easily modified in order to increase its efficiency for transferring different types of drugs. Pullulan could also be modified to be in the form of films, nanoparticles, micro-particles, micelles, and hydrogels. The therapeutic activities of pullulan include the antioxidant, anti-inflammatory, immunomodulatory, antitumor, bone regenerator, and systemic protective role, anti-glycemic, anti-lipidemic, antibacterial, and antifungal properties. The medical compatibility of pullulan-based drug delivery systems is affirmed by the profiles of experimental tests that are carried out *in vivo* or *in vitro* for monitoring their drug release and their cytotoxic effects. The recent researching challenges take into account simulative stages of the interaction between the polymeric carrying template, drug, and the residual components from pharmaceutical reagent ingredients such as the additives, surfactants, and

taste-modifying reagents. Superior and highly efficient drug carriers could deliver appropriate amounts of the drug to the targeted sites in the animal or human bodies, for keeping the concentration of the drug constant for a desirable duration, but with minimizing or without any side effects. Therefore, *in vivo* and *in vitro* cytotoxic tests for new drug delivery templates and identification of drug-release profiles are beneficial conditions for optimizing the pharmaceutical formulations.

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Chapter 12

Carrageenan-based Carriers for Therapeutic Delivery

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12.1 Introduction

Modern pharmaceutical confronts recognizing an innovative family of remedial materials and the consumption of sustainable sources to develop a polymeric material *via* economic approaches are evenly important. Polysaccharides are extensively used due to their significant applications in biomedical sciences over the last half century. Polysaccharides are an elaborated form of carbohydrate derivatives with monosaccharide subunit chains containing intermediate connections. These are normally extracted from plants, animals, and microbial sources.^{1,2} The wide accessibility and sustainability of plant biomass is taken as an emergent bio factory for the production of numerous kinds of polysaccharides. The universal sustainable biomass energy source is about 100 EJ/a and plants contributed approximately 41.6 EJ/a to this.^{3,4}

12.2 Sources of Carrageenan

The red seaweed *Chondrus crispus* was the original source of carrageenan and is also referred to as Irish Moss. As the carrageenan industry is expanding with time, there is an increasing requirement for raw materials directed toward the farming of species of *Eucheuma*, initially *E. spinosum* and *E. cottonii* and now called *Eucheuma denticulatum* and *Kappaphycus alvarezii*, respectively.⁵ The benefit of which is related to the natural *Chondrus crispus*, which is a major source of kappa- and iota-carrageenan, respectively, whereas *Chondrus crispus* comprises a combination of lambda and kappa carrageenan that is impossible to extract during industrial extraction. Hence, a major content of carrageenan is produced from *E. denticulatum* and *K. alvarezii* but many species of *Furcellaria*, *Hypnea*, *Iridae*, and *Gigartina* genera have been utilized, offering various kinds of carrageenan extracts.⁶

12.3 Extraction of Carrageenan

Two major protocols based on different principles are known for developing carrageenan. In brief, in the original and first protocol, seaweed in aqueous solution is used for the extraction of carrageenan. To eliminate the residue after filtration, usually carrageenan is recovered from solution by adding the alcohol to produce precipitation. Afterward, precipitates are separated, dried, and ground producing pure carrageenan. In the second protocol, soluble protein, residual minerals, and fat are washed out from seaweeds, leaving behind carrageenan and some other insoluble stuff. This insoluble stuff comprises cellulose and carrageenan which is then properly dried and vended as semi-redefined carrageenan that can be used for non-edible gelling applications. Though this protocol is economic and shorter than the first one and its purity is also low.^{7,8} Other techniques like fungal-treated or enzyme-treated extractions are also found in the literature.⁹⁻¹¹ For instance, enzyme-treated extraction can produce particular gelation properties, because physicochemical characteristics of carrageenan depend on the contents of the polysaccharide and the number of counter ions.^{12,13}

12.4 Production

Numerous methods have been used for the production of carrageenan from solution. The first technique is referred to as the “freeze–thaw method” in which a solution is gelled with different salts and then the gels are frozen. After thawing, water is evaporated and the remaining material is carrageenan and its salt is milled to get the required particle size. The second protocol is known as the “alcohol precipitation process” in which concentrated carrageenan solution is kept in isopropanol or other alcohols, which causes the precipitation of carrageenan. Finally, solvents are removed by evaporation and carrageenan is dried and milled to the required particle size. The third protocol is the “potassium chloride precipitation method” in which filtrate is evaporated to minimize the volume after hot extraction. Then, spinnerets are used to extrude into a cold solution of 1–1.5% KCl. The obtained gel-based threads are then rinsed with KCl solution, pressed, dried, and ground into carrageenan powder.¹⁴ The finances of the production methods depend upon the expenditures of energy required to convert the carrageenan into solution form and eventually get it in dried state.¹⁵ Industrial carrageenan is normally standardized through blending the various lots of carrageenan and including sugar or salt to obtain the required thickening or gelling characteristics (Marcel Trading Corporation, 2013). To date, major commercial sources of carrageenan are carrageenan (Viscarin[®] GP-109NF, Viscarin[®] GP-209NF), carrageenan (Gelcarin[®] GP-379NF, SeaSpem[®] PF) and carrageenan (Gelcarin[®] GP-812NF, Gelcarin[®] GP-911NF).¹⁶

12.5 Chemical Structure

Carrageenan is a sulfated polyglactean with 15–40% of ester sulfate concentration whose average molecular weight (M_w) is more than 100 kDa. It is produced by alternative units of α -galactose and 3,6-anhydro-galactose (3,6-AG) linked by α -1,3 and β -1,4-glycosidic linkage. Carrageenan is divided into different categories like λ , κ , ι , ν , μ , and θ , and all have 22–35% sulfate groups (Figure 12.1). This categorization depends upon its solubility in KCl. The differences in various classes of carrageenan which

effect its properties are content of 3,6-AG and position and number of ester sulfate groups.¹⁷ These carrageenan names do not differ in chemical structure but differ in the degree of sulfation and composition at different positions in the polymer. More ester sulfate groups mean lower gel strength, temperature, and solubility. Kappa carrageenan contains a 3,6-AG content of about 28–35% and ester sulfate contents of approximately 25–30%. Iota carrageenan contains a 3,6-AG content of about 25–30% and ester sulfate amount of about 28–30%. Lambda carrageenan contains no content of 3,6-AG and an ester sulfate amount of about 32–39%.¹⁸

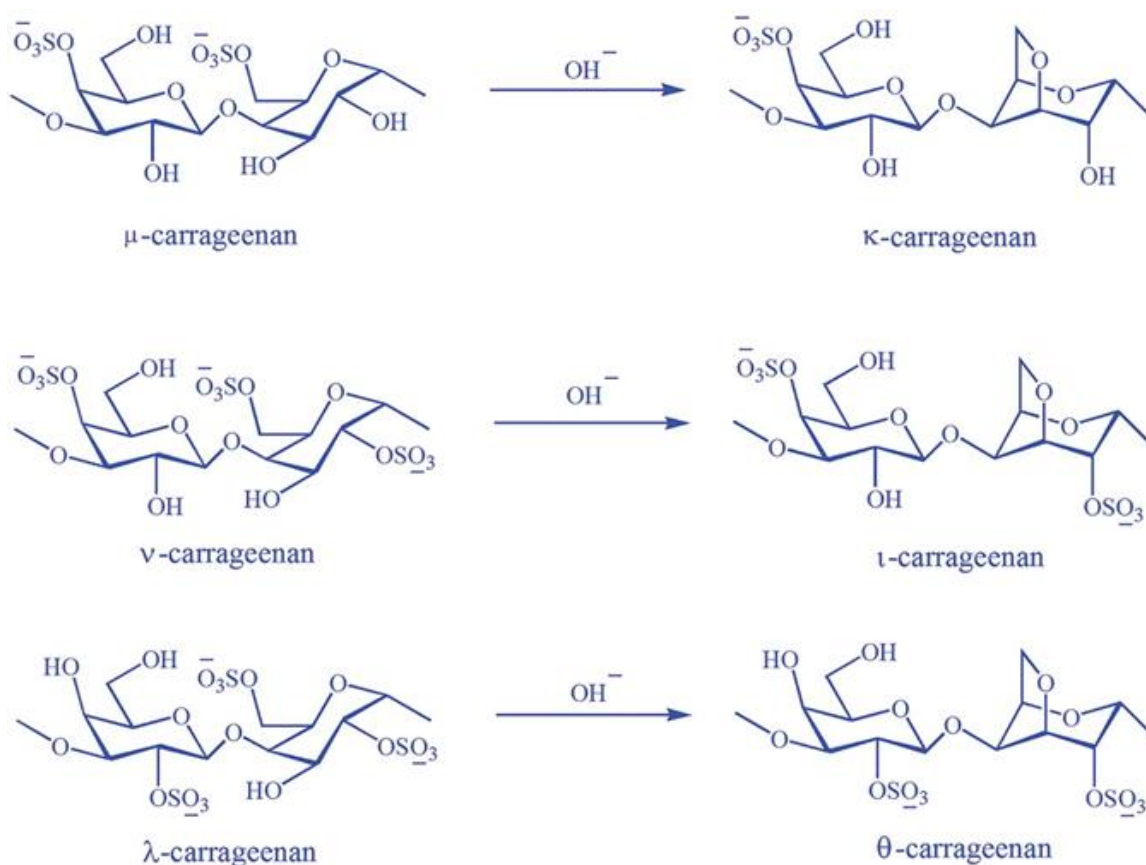


Figure 12.1 Chemical structure of different types of carrageenan.

12.6 General Properties

Commercial carrageenan has an average M_w ranging from 100–1000 kDa. Anionic sulfate groups on carrageenan have a pK_a value of around 2, which decides its degree of ionization in multiple media.¹⁹ τ and kappa carrageenan undergo thermally-induced ordered–disordered transitions, whereas chains of both types tangled like random coils with a high content of conformational entropy, at elevated temperature. Entropy is minimized and chains rearranged into more oriented conformations after cooling, which is considered to be comprised of aggregated helical, aggregated mono-helices, or double helix dimers.²⁰ τ - and kappa-carrageenan undergo gelation in the presence of di- and mono-valent cations, whereas lambda carrageenan does not undergo gelation with mono- or di-valent cations and exhibits only viscous behavior. τ - and kappa-carrageenan exhibit a three-dimensional ordered network consisting of a double helix due to the crosslinking with neighboring chains with sulfate groups arranged outwardly. On the other side, in lambda-carrageenan sulfate groups at the 2-position develop inward crosslinking and an oriented network.²¹ However, another study demonstrated that in lambda carrageenan, gelation was possible but with trivalent ions which make it possible to be used beyond a viscosity enhancing agent.²² Additionally, different salts have dissimilar influences on the phase transition and gelling of τ -carrageenan and κ -carrageenan gels. It is also mentioned that strong kappa carrageenan gels were developed with the existence of KCl salt in comparison to other salts like NaCl, LiCl, $SrCl_2$, $CaCl_2$, and $MgCl_2$.²³ In the meantime, the sol–gel transition temperature of kappa carrageenan was related to the KCl, NaCl, and $CaCl_2$ concentrations.²⁴ In τ -carrageenan, the storage modulus enhanced gradually with monovalent salt content and rapidly with divalent salt content, while in kappa carrageenan the storage modulus enhanced more quickly in the presence of KCl than with Cu or Ca. Moreover, in kappa carrageenan the storage modulus does not depend on the type and concentration of cations in more concentrated salt solution.²⁵ Carrageenan has many pharmaceutical properties like immunomodulatory, antihyperlipidemic, anticancer, and anticoagulant activities.²⁶ *In vitro* analysis showed that it has antiviral properties, preventing the repetition of hepatitis A viruses.²⁷ Furthermore, by comparing different compounds it is

also revealed that carrageenan is a very effective infection retarder for a wide spectrum of sexually encountered human papillomavirus.²⁸ It is also reported that carrageenan-based sexual lubricating gels may shield against human papillomavirus transmission.²⁹ It is also exhibited free radical scavenging and antioxidant behavior. Additionally, Rocha de Souza *et al.* reported the linear trend between antioxidant activity and sulfate content.³⁰

Carrageenan is also used as a pharmaceutical excipient and food additive. The toxicological characteristics of carrageenan are as follows: LD50 (rat, oral) $>5 \text{ g kg}^{-1}$; LD50 (rabbit, skin) $>2 \text{ g kg}^{-1}$; 4 h LC50 (rat, inhalation) $>0.93 \text{ mg L}^{-1}$. As carrageenan is employed in non-parenteral therapeutic dosages, it is considered a comparatively non-irritating and nontoxic material. When it is treated with anti-inflammatory drugs, it induced inflammatory behavior in laboratory animals,^{31,32} and hence its long-term survival is a major issue. Recent literature has indicated that prolonged induction of carrageenan in different animals caused ulcerous colonitis, promoted tumor growth and intestine mucous membrane damage. Therefore, it is crucial to undergo more essential and epidemiological experiments to investigate the safety of carrageenan.³³

12.7 Modification of Carrageenan

Carrageenan has several biomedical properties but its use in non-edible applications is restricted due to its high viscosity. Hence, carrageenan is converted into oligosaccharides by depolymerization. Different doses of gamma ray irradiation are used to degrade the carrageenan in solution, gel, or solid state at room temperature with the obtained M_w in the range of 10.5×10^4 – 0.8×10^4 with small distribution. When susceptibility to degradation and yield are compared, three categories of carrageenan in the order of $\kappa < \tau < \lambda$ are present in aqueous form.³⁴ Nevertheless, carrageenan oligomers have lower sulfate contents due to the radiation-induced desulfation. However, carrageenan can also be decomposed by microwave and ultrasound. The presence of a positive correlation between $[\eta]_n$ and k_n indicates that ultrasonic degradation of carrageenans (κ , τ) by ultrasonic waves exhibits first-order kinetics for small time spans. Ultrasound waves of low frequency and high intensity (300 W cm^{-2} , 35 kHz) caused

degradation in the sequence of τ -carrageenan < κ -carrageenan.³⁵ In the same manner, lambda carrageenan also exhibited first-order kinetics during microwave degradation at a lower microwave stress for a small duration. Susceptibility to degradation is also linked to their M_w . Additionally, microwave stresses, viscosity, conformation, concentration of polysaccharide, and stability of glycosidic linkages have remarkable effects on the degradation phenomenon.³⁶ Furthermore, other techniques for the degradation of carrageenan have also been reported in the literature such as enzymatic hydrolysis using carrageenase,³⁷ oxidative degradation,³⁸ acid hydrolysis,³⁹ and commercial enzymes.⁴⁰ The degradation phenomenon can also alter the biological functions of carrageenan. For instance, the literature revealed that M_w is a significant parameter that affects the anti-HIV (human immunodeficiency virus) functions of depolymerized carrageenan.⁴¹ Oligosaccharides from thickening carrageenan products demonstrated a much stronger tumor retarder effect⁴² and also boosted the phenomenon of wound healing. It was also reported that depolymerized carrageenan is also responsible for considerable mucosal ulceration of the colon, indicating the enhanced safety issues.³⁸ Thus, when degraded carrageenans are employed, their supplementary biological properties should also be taken into account.

Graft copolymerization can also be used to modify natural polymers which have the potential to provide the required polymeric properties. Aqueous kappa carrageenan gels demonstrate a high amount of syneresis i.e. extraction of a liquid from a gel, when undergoing aging or mechanical deformation. It is a natural phenomenon that influences the rheological and mechanical characteristics of a gel.⁴³ However, kappa carrageenan also has some drawbacks like its strong probability for microbial attack.⁴⁴ Therefore, chemical modification has been suggested to modify the physicochemical characteristics of carrageenan while grafting mostly occurred at the hydroxyl group of carrageenan. Covalent bonding of kappa carrageenan chains with other molecules can form physically more stable derivatives.⁴⁵ In the same way, bioactive properties, swelling, and specific targeting of carrageenan can also be obtained *via* grafting depending on the requirement of drug release. Additionally, other process like phosphorylated, acetylated, desulfated, and sulfated derivatives of carrageenan are also present in the

literature with numerous biological activities and physicochemical properties.^{16,46}

12.8 Carrageenan-based Carriers

12.8.1 Hydrogels

Hydrogels are 3D hydrophilic crosslinked polymer networks that can retain a high amount of water, physiological, or saline solutions.⁴⁷ Natural biopolymers like polysaccharide-based hydrogels have gained remarkable attention owing to their renewability, biocompatibility, safety, and biodegradability as compared to the synthetic polymer-based hydrogels. Due to numerous characteristics like high water-holding capacity and strong gel-forming ability of carrageenan, it is used in various biomedical applications and considerably in drug delivery.^{48–51}

The influence of rod-shaped and spherical gold nanoparticles on the drug release and thermomechanical properties of kappa carrageenan hydrogels was determined and demonstrated that gold nanoparticles reinforce the hydrogel structure. These influences on the strength and microstructure of hydrogels suggest the sustained release phenomenon as explained by *in vitro* study employing a model drug. Kappa carrageenan hydrogels filled with gold nanoparticles not only contained optical features that can modify its morphology by fillers but also their drug carrier behavior that can be modulated by tuning the characteristics of gold nanoparticles.⁵² It is also reported in the literature that the synthesis and characterization of iota carrageenan-based hydrogels reinforced with biodegradable silver nanoparticles act as striking antibacterial agents. The authors concluded that silver nanoparticle-based hydrogels were efficient for antimicrobial fields.⁵³

The physical properties of the carrageenan modified with the addition of genipin are studied for the controlled release of drugs. This modulation caused relaxation in the chains of carrageenan molecules and increased swelling which creates the sustained delivery of β -carotene in the presence of genipin.⁵⁴ In another study, kappa carrageenan and polyvinyl alcohol crosslinked hydrogels were developed in the presence of genipin as a non-toxic and natural crosslinker to attain sustained drug delivery. It is

demonstrated that genipin can manage the rapid delivery in hydrogels and control the active substance better than native gels due to their structural modulation. By changing the crosslinker ratio, morphological changes in polymer network also occur which ultimately affect the release rate. This inferred that the release rate is related with the crosslinking degree and mesh size offered for drug diffusion.⁵⁵

Kappa carrageenan, hyaluronic acid (HA), and epichlorohydrin (crosslinker)-based hydrogels were investigated. These hydrogel samples were entrapped with l-carnosine as a model drug. HA affects the water uptake characteristics and release rate, as the cumulative release significantly increased from 28 to 93% with increasing HA content. By increasing the HA content rather than kappa carrageenan, a slight change in tensile strength occurred, whereas a maximum strain value for kappa carrageenan-based hydrogels was 498.38% with an HA content of 3%. The thermal properties of the kappa carrageenan and HA blended hydrogel were better than that of pure HA. The authors deduced that these hydrogels can be employed in various biomedical applications.⁵⁶

Mahdavinia *et al.* described the controlled release of ciprofloxacin using chitosan/kappa carrageenan/hydroxyapatite complexes. The ciprofloxacin-loaded nanocomposite hydrogels showed antibacterial behavior against *E. coli* i.e. Gram-negative and *S. aureus* i.e. Gram-positive bacteria. Hydroxyapatite caused the sustained release of ciprofloxacin. The pure chitosan/kappa carrageenan hydrogel released 98% of ciprofloxacin in 120 h, while the chitosan/kappa carrageenan/hydroxyapatite-loaded hydrogel showed the release as 52 and 66% with a high and low concentration of hydroxyapatite, respectively. Controlled delivery of ciprofloxacin from the nanocomposite complex confirmed that they are a striking material for the drug release system with controlled delivery behavior.⁵⁷

Rasool *et al.* fabricated a carrageenan/polyvinyl alcohol stimuli active hydrogel with a silane-based crosslinking agent. All specimens demonstrated efficient antibacterial behavior against *S. aureus* and little activity against *E. coli*. Cephadrine release was enhanced with rising pH and time, and 85.5% of cephadrine was delivered in 7.5 h in a controlled way as explained by *in vitro* study. The authors claimed that these hydrogels can be a striking material for an injectable drug release system.⁵⁸ In another

study, researchers fabricated a pH responsive hydrogel comprised of carrageenan, polyethylene glycol, and sodium alginate in the presence of a crosslinker *i.e.* 3-aminopropyl triethoxysilane, demonstrated that the developed hydrogel had a sustained delivery of lidocaine with excellent cell compatibility. Hence, it can be employed for a successful sustained drug release system.⁵⁹

Along with other applications, in another study, an oral sustained delivery of carbamazepine gel was designed for pediatric use. In this study, iota carrageenan encapsulated with carbamazepine alginate beads was formulated and the syneresis, appearance, rheology, stability of the final hydrogel, uniformity of the drug amount, and release behavior were investigated. The hydrogel showed better shear thinning characteristics, no syneresis, and good homogeneity. The release behavior of the hydrogel and beads were not considerably different and was the same as for the controlled formulation. Consequently, the physical stability results showed that this hydrogel formulation can sustain its characteristics for one month upon saving at 30 °C. The researchers acknowledged that carbamazepine in a hydrogel-controlled delivery dosage form gathered the benefits of tablet form regarding sustained release profile and benefits of suspension form in terms of the dosing flexibility.⁶⁰

In this research, mupirocin and ketoprofen-based microparticles were entrapped in the kappa-carrageenan/locust bean gum hydrogel to develop a material as a dual drug release carrier. The hydrogels demonstrated a slow release, approaching the time span of 7 days at 37 °C, compared with microparticles and hydrogels individually for both drugs. Therefore, these types of hydrogels can be regarded as a possible vehicle for poorly water-soluble drugs, particularly for applications in wound healing.⁶¹

This study showed that kappa carrageenan has the ability to boost the drug release performance and physicochemical properties of agar hydrogels. Different hydrogels with varying weight ratios of kappa carrageenan and agar were evaluated. The results indicated that the gel melting temperature, gelling temperature, and gel strength decreased with increasing contents of carrageenan but apparent viscosities enhanced. These formulations were also studied as a carrier for the release of metformin hydrochloride. The sustained release capacity and drug loading efficiency

can be improved by increasing the kappa carrageenan contents for 9 h. It is also notable that the release behavior normally depends upon the electrostatic interaction between polysaccharides and the drug.⁶²

12.8.2 Tablets

Hydrophilic polymer matrix systems are firstly used drug release systems for controlled release. Owing to the favorable physicochemical characteristics of carrageenan, they are employed as matrices for encapsulating the drugs with low bioavailability. The effectiveness of lambda carrageenan and iota carrageenan for the formation of controlled released tablets was evaluated. Chlorpheniramine maleate, sodium salicylate, and theophylline were employed as sample drugs. 500 mg matrices with a diameter of 0.5" were used. They used carrageenan with a drug of two different ratios 13.5% and 16.9% and two dissimilar diameters of 0.375" and 0.5" on release behavior. The results demonstrated that matrices with carrageenan were helpful to develop controlled release between 8–12 h, while the release profile followed zero-order kinetics.⁶³ The drug to carrageenan ratio, tablet diameter, and ionic strength of the dissolving media play a major part in drug delivery. Therefore, the drug delivery rate enhanced both with the drug to carrageenan ratio and tablet diameter. These parameters should be taken into account during preparation of controlled drug release formulations with carrageenan.⁶³

Buchholz *et al.* prepared modulated released iota carrageenan-based matrix tablets mixed with lactose and microcrystalline cellulose in various ratios, with riboflavin 50-phosphate sodium (vitamin B2) as a dynamic model drug. The dissolution response was checked in phosphate buffer (pH 4.5) and gastric fluid (pH 1.2) with the result of preparation of sustained delivery tablets. The release behavior demonstrated a linear increase up to 80%. Lactose affected the response of the matrix; as the elasticity of the matrix was increased with a decreasing amount of lactose, and ultimately developed tablets with poor mechanical strength.⁶⁴

Lambda carrageenan with two model drugs, metoprolol tartrate and diltiazem HCl, was investigated in matrix tablets. These two formulations release the drug through different mechanisms, showing two different polymer–drug interactions. Carrageenan/diltiazem developed a slightly

soluble slow dissolving matrix, whereas carrageenan/metoprolol tartrate was a poorly stable matrix, producing an erodible matrix in only 8 h. The researchers conclude that these formulations show two different drug release systems; metoprolol tartrate adopts the classical soluble matrix type release system while in diltiazem hydrochloride, diffusing/dissolving specie is itself complex because of very strong contact between the polymer and drug. In such situations, tablets must be taken as a monolith comprised by a single insoluble compound rather than a matrix system.⁶⁵ In this research, the release protocol of doxazosin, which is a positively charged drug, from tablets based on lambda carrageenan is reported. The trials were conducted with different ionic strength in solution form by the inclusion of ionic surfactant *i.e.* sodium dodecyl sulfate (SDS). Results demonstrated that release behavior strongly depends upon the polymer–drug interaction. The amount of SDS below critical micelle concentration (CMC) slows down the drug release due to the hydrophobic binding of SDS with a polymer/drug complex, whereas at a concentration above CMC, it withdraws the drug by developing mixed micelles with it, and hence boosts the release.⁶⁶

Yermak *et al.* investigated various categories of carrageenans as matrices loaded with echinochrome A to improve and protect drug dissolution. The loading of echinochrome in carrageenan augmented echinochrome solubility, reduction in its oxidative degradation, and changes in size, charge and morphology of carrageenan. Carrageenan loaded with echinochrome also demonstrated mucoadhesive characteristics. The release behavior of echinochrome based upon the chemical structure of carrageenan and specific ions. The researchers showed that behavior of the echinochrome-loaded carrageenan is better than pure drug. Therefore, novel carrageenan-based tablets are suitable for oral administration and extend the release of echinochrome.⁶⁷

Another novel pharmaceutical application which is vaginal tablets has been explored. Kappa carrageenan-based vaginal tablets used for acyclovir sustained release were evaluated. In this study, drug release was checked by two methods; first in simulated vaginal fluid and second in the mixture of simulated vaginal and simulated seminal fluid mixture. *In vitro* biocompatibility, bioadhesion resistance time and bioadhesive capacity were also investigated. These tablets demonstrated the extended delivery of

the drug for a time span of 7 days. It was also reported that kappa carrageenan with hydroxypropyl methylcellulose (HPMC) was also important for controlled delivery. The residence time of kappa carrageenan and HPMC formulation was between 72 and 108 h. The results demonstrated that kappa carrageenan had zero cytotoxicity at the highest used content in terms of biocompatibility, and might be useful for the inhibition of genital herpes infection.⁶⁸

In another advanced research study, vaginal tablets comprised of iota carrageenan and HPMC were fabricated and investigated. This formulation attained the sustained delivery of acyclovir in 96 h. These vaginal tablets were demonstrated to be useful to inhibit the sexual transmission of genital herpes, because the microstructure of the polymer matrix has a high mucoadhesive capacity and appropriate swelling rate, which leads the formulation to stay in the vaginal area until the complete release of acyclovir.⁶⁹

12.8.3 Beads

Beads based on carrageenan have also been reported as drug delivery systems. The fabrication of mefenamic acid-based beads, a non-steroidal anti-inflammatory drug comprised of kappa carrageenan, was described which was intended to minimize the daily dose and decrease the gastrointestinal disorders due to the conventional medicine.⁷⁰ Carrageenan mixed with chitosan makes the polyelectrolyte complex and has been reported as a sustained release for diclofenac sodium. The results indicated that the delivery rate of diclofenac sodium can be modulated for a long period using a carrageenan to chitosan ratio of 1 : 2 with a drug concentration of 5% (w/v). The glutaric acid-based crosslinked beads demonstrated the improved properties compared to beads fabricated without a crosslinker. The glutaraldehyde-based crosslinked beads were better for sustained delivery of drug for 24 h. It was noticed that diclofenac delivery was slower at pH 1.2 and 6.8 and quicker at pH 7.4, providing an efficient sustained delivery system in the administration of particular colon-targeted drugs.⁷¹

In another study, polyacrylamide grafted on sodium alginate and kappa carrageenan-based pH sensitive hydrogel beads were reported to target

ketoprofen toward the intestine. The beads demonstrated a strong pH response in pulsatile swelling property. The delivery of ketoprofen was considerably enhanced upon changing the pH from acidic to basic. The beads demonstrated 10% drug release at acidic pH while approximately 90% release in basic medium of 7.4 pH. The histopathology of the stomach of albino rats demonstrated that gastric side effects, like erosion of the gastric mucosa, hemorrhage, and ulceration were minimized upon loading of drug into pH-stimulated hydrogel-based beads.⁷²

The development of kappa carrageenan-based pH responsive and magnetic beads for drug targeting carriers is also reported. The results indicated that the swelling response of beads at different pH values was lower when the kappa carrageenan concentration was increased. Riboflavin was induced in hydrogel beads and the drug release response indicated that drug release was higher at pH 7.4. The slowest delivery behavior at a lower pH was noticed for hydrogel beads comprising only an alginate constituent, which showed that the response was due to the pH sensitive behavior of sodium alginate.⁷³ In one more study, researchers reported the fabrication of carboxymethyl chitosan and kappa carrageenan magnetic beads used for the drug release system with diclofenac sodium as a model drug. Results revealed the pH-dependent behavior for *in vitro* drug release. The accumulative release was low at a pH of 1.2, while high at pH 7.4 for all prepared beads. Researchers reported that inclusion of magnetite nanoparticles considerably affected the drug delivery pattern and the action of beads toward external stimuli make them striking candidates for novel drug release systems.⁷⁴

Carrageenan-based solid lipid pearls demonstrated the ability to coat the bitter taste of enrofloxacin and extend its delivery rate.⁷⁵ In this research, pectin/alginate microbeads loaded with caffeine confirmed that the use of biopolymers like chitosan, psyllium, and carrageenan and the inclusion of caffeine can positively modulate the perception of bitterness.⁷⁶ Kappa carrageenan-based bionanocomposite hydrogel beads and bio-synthesized silver nano particles were characterized as an antimicrobial material. Kappa carrageenan silver nanoparticle beads demonstrated strong antimicrobial activities against *E. coli*, *P. aeruginosa*, methicillin-resistant *S. aureus*, and *S. aureus* with the highest zone of inhibition (11.2 mm). The cytotoxicity

study demonstrated that kappa carrageenan with silver nanoparticle-based nanocomposite hydrogels exhibited a satisfactory toxicity level and high pharmacological ability owing to their acceptable safety level to be used in biomedical applications.⁷⁷

Kappa carrageenan-based beads produced as carriers for administering curcumin in cancer cells achieved drug delivery under a pH responsive environment (5 and 7.4). Curcumin was successfully loaded in kappa carrageenan and resulted in an improved ability to effectively load and release in a controlled manner. *In vitro* controlled release of curcumin occurred at pH 5. The cytotoxicity of curcumin-loaded kappa carrageenan beads had a remarkably high apoptotic activity in chosen lung cancer cells of A549. The researchers recommended that curcumin-encapsulated kappa carrageenan can be a striking material for producing a polysaccharide-based natural release carrier with boosted remedial activity for clinical systems.⁷⁸

12.8.4 Suppositories

Microbiocide research is presently increasing due to its benefits for the inhibition of sexually transmitted tenofovir (TFV) as an antiviral agent. *In vitro* dissolution analyses in aqueous medium, semen stimulant fluid, and vaginal stimulant fluid indicated that approximately 45–50% of TFV was released from the suppositories within 2 h in both media, regardless of volume and size.⁷⁹ In this study, Zaveri *et al.* reported the development of semisolid suppositories by using a combination of Carbopol[®] 940P and carrageenan along with TFV. The results demonstrated that about 45–50% of TFV was delivered in the first 2 h, 60% in 6 h, and 70% in 24 h. There was no considerable difference in TFV delivery rate of different gels of Carbopol[®] and carrageenan or in the amount of TFV release in 48 h. The researchers found that the material with high Carbopol[®] exhibited an improved acceptability rate; as the size, shape, properties, and firmness of the product after insertion affect the application of these microbiocides. Hence, a combination of Carbopol[®] with kappa carrageenan significantly augments its acceptability.⁸⁰

12.8.5 Oral Suspensions

Ionic interaction of drugs with carrageenan causes lower drug dissolution retardation and solubility which may be employed to fabricate the controlled delivery dosage form of drugs which are water soluble. Bani-Jacer *et al.* developed a combination of ambroxol with a carrageenan complex and raft formation to get a controlled release liquid suspension of ambroxol (ABX). The material was developed in the form of suspension in an aqueous raft forming vehicle of calcium carbonate and sodium alginate. An ABX or ABX complex-based suspension demonstrated rapid raft formation and raft flotation in dissolution medium. The suspension of ABX–carrageenan complex demonstrated better control over ABX release than the free drug suspension. The drug delivery from the suspension was dual phasic with the first rapid delivery followed by a controlled delivery phase with a slow drug delivery rate. The release rate of the complex suspension over the free drug suspension can be ascribed to the fact that one more step *i.e.* dissociation of the ABX–carrageenan complex was demanded for the release before drug dissociation. The authors observed that dual phasic delivery of ABX could be beneficial to cure chronic and acute respiratory diseases along with enhanced mucus production; a quick relief from the accompanying annoying symptoms is a priority. If relief was set for an extended time, a lower dosage frequency would be needed.⁸¹

12.8.6 Pallets

Literature reports demonstrate that kappa carrageenan is a new material helpful for pallet formation with process robustness and development.^{82,83} Kappa carrageenan-based pallets undergo rapid disintegration in comparison to others, like microcrystalline cellulose pellets, which is linked with fast drug delivery especially for slightly soluble active components.⁸⁴ Burst drug delivery from kappa carrageenan can be useful in the preparation of enteric coated pallets using this procedure. Ghanam *et al.* evaluated the viability of developing multi-particulate tablets with coated kappa carrageenan pellets. The results indicated that kappa carrageenan granules are more beneficial than microcrystalline cellulose granules for the preparation of multi-particulate tablets with enteric characteristics. Kappa carrageenan granules can also be designed in multi-particulate tablets with controlled delivery characteristics.⁸⁵

In this study, HPMC K15 M, kappa carrageenan, microcrystalline cellulose, and nifedipine granules were prepared for controlled mucoadhesive release systems. Results indicated that kappa carrageenan has the ability to be used for the development of pellets with the required flow, sphericity, hydrogel developing abilities, and size, for robust pellet fabrication. Kappa carrageenan, microcrystalline cellulose and HPMC K15 M in the ratio of 20 : 35 : 10 w/w can be used as an efficient system for improving the controlled release and sphericity of matrix pellets, which indicated that the mixture with other polymers enhanced the preparation parameters.⁸⁶

In recent study, pellets were prepared without using microcrystalline cellulose to prevent the drug incompatibility and poor disintegration of the granule matrix. Researchers developed the pellets using chitosan as a diluent, kappa carrageenan as a spheronization aid, and Carbopol[®] 974P as a binder for the fabrication of pellets along with acetaminophen as a model drug. Results of the developed pellets indicated the acceptability of developing good quality pellets without microcrystalline cellulose. The pellets were robust and spherical and approached the criteria of burst release.⁸⁷

12.8.7 Internasal Systems

Iota carrageenan indicated an antiviral response against influenza A virus and human rhinoviruses when used *via* a nasal route and numerous researchers have reported the antiviral behavior.⁸⁸⁻⁹¹ The non-pharmaceutical safety of internasal iota carrageenan has been investigated. Experiments on animals including toxicity and repeated local dose tolerance analysis studied 0.12% iota carrageenan intranasally for 7 to 28 days in New Zealand white rabbits and the rats were nebulized to F344 rats for 7 days with 0.12% iota carrageenan administration. The results demonstrated no confirmation of toxicity and local intolerance when carrageenan was used *via* inhalation or intranasally. Therefore, it was concluded that 0.12% iota carrageenan is sufficient for pharmaceutical applications for intranasal applications.⁹² Safety evaluation of iota carrageenan permitted the fabrication of new developments for nasal applications. Kappa carrageenan and chitosan-based polyelectrolyte complexes for nasal inserts were

developed to improve the therapeutic efficiency of sumatriptan succinate. The molar ratio of polycations and polyanions imparts a major role to tune the properties of nasal inserts. A kappa carrageenan and chitosan ratio of 4 : 1 showed excellent mucoadhesive ability and water uptake potential and offered sustained delivery of sumatriptan succinate, making this material a striking release system for sumatriptan succinate for the curing of migraine attacks.⁹³

12.8.8 Micro/nanoparticles

Fabrication of carrageenan-based nanoparticles has also been developed by many researchers.⁹⁴ A few years ago, carrageenan/chitosan-based nanoparticles used for drug delivery were comprehensively evaluated. They are normally formulated *via* the formation of polyelectrolyte complexes and ionic gelation by combining cationic polymers with carrageenan.⁹⁵ The ability of chitosan, lambda carrageenan, iota carrageenan, and kappa carrageenan to prepare a controlled delivery system for glucose oxidase was outlined in the literature. Chitosan/carrageenan complexes with charge ratio of 3 and 5 can entrap glucose oxidase and modulate its release. Protein is loaded into the complex through the mechanism of electrostatic interaction, and swelling phenomenon caused the controlled release of glucose oxidase from the complex. Authors claim that chitosan/carrageenan complexes are potential drug release systems to be used for oral administration of proteins and peptides.⁹⁶

Grenha *et al.* reported the development and analysis of protein-encapsulated nanoparticles achieved *via* ionic complex formation of chitosan/carrageenan as a drug release system. Chitosan/carrageenan-complexed nanoparticles tendered a controlled and sustained release of protein for 3 weeks by *in vitro* release assay. These nanocarriers also demonstrated low toxicity when in contact with fibroblast-like cells, which is a promising sign of their safety and biocompatibility.⁹⁷

Chitosan/carrageenan/tripolyphosphate (TPP) nanoparticles were fabricated *via* ionic gelation/polyelectrolyte complexation. TPP served as a crosslinker and caused the formation of smaller size nanoparticles along with an increase in production yield. The inclusion of TPP in a nanoparticle matrix enhanced their stability up to 9 months. Researchers indicated that

charge ratio plays a vital part in the fabrication of nanoparticles, as a charge ratio around 1 caused the precipitation means neutralization of charges, whereas a high charge ratio did not offer sufficient charges to permit an interaction that causes nanoparticle development. The nanoparticles are striking candidates in the applications of mucosal release of macromolecules owing to their positive load and small size.⁹⁸ In another study, they explained the efficacy of chitosan/carrageenan/TPP nanoparticles as vehicles for use in nasal and pulmonary drug release. The nanoparticles were stable along with the specific concentration of lysozyme simulating an *in vivo* environment. The carriers showed negligible or low cytotoxicity in contact with two respiratory cell lines presenting both the pulmonary and nasal epithelium, as represented by the effects on transepithelial electrical resistance and cell metabolic activity.⁹⁹

Rosas-Durazno *et al.* prepared the prototype of nanocapsules of kappa carrageenan polyelectrolyte with a dodecyl-trimethylammonium chloride stabilized nanoemulsion by electrostatic interaction. The researchers observed that interaction of kappa carrageenan with the nanoemulsion developed the thermo-reversible coil to helix transition characteristics of kappa carrageenan and other similar polysaccharides to fabricate temperature stimuli nanocarriers. The inclusion of cationic polymer in nanoemulsion also caused the reduction in cytotoxicity.¹⁰⁰

Nano-microparticles loaded with curcumin were developed to boost the stability and solubility of curcumin under gastrointestinal conditions. These nano-microparticles were prepared by using carrageenan, chitosan, and alginate. Results indicated that nano-microparticles prepared with a carrageenan and alginate ratio of 50 : 50 at pH 7.4 released more than 90% of curcumin in 7 h. Carrageenan imparts a vital role for the improvement of curcumin release from a hydrogel matrix, because a high concentration of carrageenan caused more release of curcumin.¹⁰¹

Carboxymethylated kappa carrageenan microparticles functionalized with lectin with insulin loading were investigated by *in vitro* and *in vivo* analyses. This loaded insulin was prevented from proteolysis and hydrolysis by enzymes and stomach acids. When this entrapped insulin was orally administered to diabetic rats, it showed an extended hypoglycemic influence for up to 12–24 h. Results concluded that these prepared

microparticles were used in the fabrication of oral insulin release systems.¹⁰²

Polyelectrolyte nanocapsule complexes of chitosan and kappa carrageenan were fabricated by loading of neem seed oil. Neem seed oil was removed from the nanocapsules and then isoniazid was encapsulated. The drug was encapsulated at a fast rate at the initial stage and then at a slow rate. The release of isoniazid based upon the pH of the medium was determined, as it was slower in basic medium and higher in acidic medium.¹⁰³ Bosio *et al.* describe the development of hybrid nanoporous microparticle carriers comprised of biopolymers functionalized with folic acid (FA) and calcium carbonate, and with doxorubicin as a chemotherapeutic model drug. The modification of lambda carrageenan with FA in the microparticles enhanced the cancer cell targeting and also increased the particular surface area. Controlled drug delivery was observed under a simulated physiological environment for lambda carrageenan and drug entrapped microparticles in 25 days experiments. The anticancer response of these drug-loaded microparticles toward the human osteosarcoma MG-63 cell line demonstrated cell viability of 100 and 13% without and with doxorubicin, respectively. This recipe is new for the chemotherapy of cancer.¹⁰⁴

In this study, maghemite (Fe_2O_3) nanoparticles were added in iota carrageenan to fabricate a new material. Results showed the interaction of nanoparticles with the sulfate groups of carrageenan. These nanoparticles can be used in biological systems due to their characteristics. By varying the ratio of iota carrageenan and nanoparticles, its surface properties can be changed to make them useful for the treatment of cancer cells. Their potential to retard the growth of cancer cells without harmfully affecting normal cell lines make it a potential nanovector to be used in drug release.¹⁰⁵

Magnetic chitosan/kappa carrageenan carriers were prepared for the release of sunitinib behaved as a potential material for a pH responsive smart drug delivery system. These magnetic carriers showed the acceptable rate of drug encapsulation and permitted the controlled release of sunitinib. The drug was completely released from nanoparticles in 7 days. Researchers confirmed that these magnetic carriers can be used in cancer

therapy, because its sunitinib release was pH-dependent and lowest at 7.4 pH, so magnetic carriers may be potential materials for anticancer therapeutics with minimum side effects.¹⁰⁶

In another study, kappa carrageenan-coated zinc oxide nanoparticles were fabricated and their behavior as an antibacterial material against infections was investigated due to the microorganisms resistant to various drugs. Nanoparticles considerably retarded the development of biofilm at low content and minimized the hydrophobicity of the bacterial cell surface. No morphological changes in red blood cells and hemolysis were observed. The nanoparticles were non-toxic toward NIH3T3 mouse embryonic fibroblast cells and no viability changes were recorded up to 500 g mL^{-1} . Ecosafety evaluation with *Artemia salina* demonstrated that the nanoparticles were not toxic to the environment. These results indicate a nano-anti-superbug drug with extensive properties that are useful as nano antibiotics to cure various ailments.¹⁰⁷

Polysaccharide-based aerogels attain the characteristics of both polysaccharides and aerogels, which permits them to be used in different systems. In this perspective, Alnaife *et al.* described that the microparticle aerogel could be used as a biocompatible polymer. Carrageenan-based microspherical gel particles were prepared *via* emulsion technology. A supercritical carbon dioxide extraction technique was used to transform the gel into an aerogel. The results showed that the surface area of the aerogel was in the range of $33\text{--}174 \text{ m}^2 \text{ g}^{-1}$, and the average pore size and pore volume were $12.34\text{--}3.24$ and $0.35\text{--}0.11 \text{ cm}^3 \text{ g}^{-1}$, respectively. This porous material therefore demonstrated promising characteristics to be used in drug delivery applications.¹⁰⁸

In this study, Obaidat *et al.* designed carrageenan spherical aerogel microparticles as a promising drug vehicle. Ibuprofen was chosen as a reference drug and microparticles were developed using an emulsion gelation technique and can be used as a drug carrier. Ibuprofen was effectively entrapped in amorphous form in the prepared microparticles with remarkable improvement in drug delivery response.¹⁰⁹

12.8.9 Wafers

Kianfar *et al.* prepared wafers based on pluronic acid, kappa carrageenan, and polyethylene glycol 600 loaded with a model soluble drug of paracetamol and insoluble drug of ibuprofen for buccal release. These ingredients were taken in the following concentrations: pluronic acid 4% (w/w): kappa carrageenan 2% (w/w), PEG 600 4.4% (w/w), ibuprofen 0.8% (w/w), and paracetamol 1.8% (w/w). The authors confirmed that these buccal wafers showed an excellent release profile in simulating conditions of saliva which was combined with their required mucoadhesive properties that can be used for buccal drug release systems.¹¹⁰

In this research, Pawar *et al.* prepared the wafers using polyox, along with sodium alginate or carrageenan with diclofenac sodium and streptomycin to enhance the chronic wound healing properties. Wafers demonstrated sustained delivery of diclofenac sodium and streptomycin due to different pore sizes and the preparation of sodium sulfate because of the salt forms of these two drugs. The authors recommended that wafer can be efficiently employed for exuding wounds like chronic ulcers.^{111,112}

12.9 Applications of Carrageenan in Therapeutic Delivery

12.9.1 Oral Delivery

The oral route of drug delivery systems is widely used because it has greater patient ease. It is important to synthesize a drug release system which can maintain stability at high pH in the gastrointestinal region. Carrageenan is extensively used for microencapsulation of numerous probiotics, as a stabilizer in the food industry and as food additives.^{113–115} Modification of *L. plantarum* increases the persistence in simulated intestinal fluid because it is sensitive toward acidic pH. Swelling and erosion in the matrix at simulated intestinal fluid are directly proportional to the discharge of probiotic bacteria into carboxymethyl cellulose/kappa-carrageenan and quick release was detected. Therefore, this system was used as an oral delivery carrier to the colon.¹¹⁶ *Lactobacillus rhamnosus* is another probiotic Gram positive bacterium. Nanoparticles of clay have progressively been used for their biomedical properties and achieved

consideration because of the thermal and mechanical constancy of these nanoparticles.^{117,118} Halloysite nanotubes (HNTs) induced into κ -carrageenan hydrogels not only made it biocompatible but also increased their thermal and mechanical properties and drug release profile. Improved mechanical and thermal strength due to contact with polymer and HNTs was hypothesized because of framing of carrageenan yields in the lumen of HNTs. Rather than swelling, drug particle charge also affects the discharge rate. Anionic dye, orange G, and rhodamine B, a cationic dye were selected as reference drugs for encapsulation into HNTs. Electrostatic interaction was developed for incorporation of negatively charged anionic drugs, whereas in the case of cationic drugs the repulsion between the drug and HNTs becomes the reason for faster release. Thus, electrostatic repulsion due to accumulation of HNTs improved the drug-loading capacity as well as the drug-release rate.¹¹⁹

A nanogel was designed as an oral drug delivery system by using κ -carrageenan modified with low-density lipoprotein (LDL), whereas curcumin was used as a reference drug in the system by means of hydrophobic contact. Nano-gels presented greater constancy in gastric and intestinal surroundings. LDL/ κ -carrageenan-based nano-gels showed sustain and slow release kinetics in both gastric and intestinal environments, so appear to be the most favorable for oral drug delivery systems.¹²⁰ Metal oxide and its nanoparticles have a greater surface area such as magnesium oxide (MgO) as well as silver nanoparticles scientifically considered for their biomedical importance due to their exceptional physiochemical properties.¹²¹

To develop Ag nanoparticles, κ -carrageenan hydrogels when achieving their swelling equilibrium point were initially dipped into AgNO₃ solution so that the silver ions attached onto the surface and then compacted with sodium borohydride. To build an in situ combination among hydrogel and metallic nanoparticles and to attach the maximum number of ions, the hydrogel must have excellent swelling properties in the mixture of carrageenan and carboxymethyl cellulose.¹²²

The drug release pattern was examined in a replicated gastrointestinal situation with methylene blue as a sample drug. The hydrogel showed the good release profile of methylene blue drug as with the increase in Ag

nanoparticles the osmotic pressure rises and results in an incessant influx of water. In an intestinal situation, as crosslinking triggered higher absorption of genipin, it resulted in size reduction of the nanoparticles with continuous discharge in acidic environments.

Similarly, metal oxide nanoparticles functioned as a source for methylene blue absorption in κ -carrageenan-sodium carboxymethyl cellulose hydrogels and more methylene blue discharge was observed than with the control hydrogel. Thus, κ -carrageenan hydrogels with metal and metal oxide nanoparticles offered amazing controlled oral drug delivery systems in both gastrointestinal environment conditions.¹²³

12.9.2 Ophthalmic

Stimuli-sensitive hydrogels are a commonly used form in the last few decades in ocular and ophthalmic inventions.¹²⁴ κ -Carrageenan, alginate, and gellan gum-based hydrogels were prepared with varying concentrations of biopolymers, and their ionic interactions and in situ gelling properties were studied.¹²⁵ κ -Carrageenan–gellan gum hydrogels were observed using positron emission tomography/computed tomography (PET/CT) along with fluorine radiotracers (^{18}F) such as ^{18}F -NaF and ^{18}F -FDG and showed a persistent corneal contact time related to the aqueous media. However, measuring the approval of hydrogel from the ocular surface with the help of methods like MRI and PET/CT was quite problematic. Scintigraphy was used to observe the physiological procedures such as the discharge system and tear drainage.¹²⁶ The approval of hydrogels was determined with the technetium pentetate ($^{99\text{m}}\text{Tc}$ -DTPA) radiotracer which presented one-phase decay kinetics. Thus, using a smaller quantity of radioactive elements in ophthalmic preparations observed safe for ocular pharmacokinetic assessments. At 60 °C methylcellulose converted into a sol–gel and to slow down this conversion at ambient temperature κ -carrageenan was added as it disturbs the interaction among –OH of methylcellulose.¹²⁷

12.9.3 Nasal Delivery

Enhanced patient comfort, and greater permeability and capability to avoid first pass metabolism has prolonged the use of nasal drug delivery

systems.¹²⁸ Approval of nasal transport medium,¹²⁹ partial moistening of nasal supplements,¹³⁰ less availability of micro and nanoparticles, and inadequate medicine packing or discharge¹³¹ are great obstructions to the transformation of nasal drug release schemes. Hydrogel systems established for intranasal transfer initially when two polymer solutions come in contact with each other they still remain liquid and gelled in situ. Therefore, poloxamer hydrogels cut down into fragments in solution, and the resulting in situ gel was formed. Ketorolac tromethamine was attached with a poloxamer. Drug release from the poloxamer was moderately lower as associated with the control pH and no nasal ciliotoxicity was detected. Therefore, the amount of K^+ ions is an essential character in the breakdown of hydrogels; the greater the concentration of K^+ ions, the smaller the erosion process.¹³²

12.9.4 Transdermal

The transdermal route of drug delivery is the topical, painless, and systematic method where drug is absorbed through skin into the bloodstream and remains free from degradation due to enzymes and pH.^{133–135} Some common ways to deliver drug through the transdermal route are iontophoresis and transdermal patches but hydrophilic drugs cannot integrate into the skin due to its hydrophobic nature. To develop a hydrogel blend κ -carrageenan was mixed with an electroconductive polymer, polythiophene, and loaded with the model drug acetylsalicylic acid. The hydrogel mesh size decreases with the increase in concentration of crosslinker due to the effect of crosslinking ratio. As compared to smaller ions, larger ions ($Ba^{2+} > Ca^{2+} > Mg^{2+}$) upon electrical stimulation facilitate more drug diffusion as they relax the carrageenan network and bring about a change in carrageenan microstructure. The transdermal delivery rate of drugs is affected by the M_w of ions and the existence of polythiophene.¹³⁶ One commonly used very effective skin protective compound against damaging UV rays is tocotrienol rich palm oil-based vitamin E (TRPE).¹³⁷ A semi interpenetrating network of hydrophobic TRPE and hydrophilic polymer was prepared with the help of surfactant in the form of emulsion (oil in water).

Carrageenan, locust bean gum, and guar gum were initially mixed with glycerin until a uniform gel was obtained at high temperature. To mix TRPE the solubilizer used is hydrogenated castor oil, PEG-40, and potassium citrate is used as a preservative. The gel flexibility decreased but the strength was increased with the further addition of potassium citrate and guar gum, whereas glycerin was added to increase in the flexibility. The gel permeation ability improved because of the emulsification property of PEG-40 and castor oil, thus making these gels non-irritant, biocompatible, and biodegradable with excellent flexibility as well as strength.^{138,139}

12.10 Conclusion

The applications of carrageenan in therapeutic fields are increasing drastically day by day. It is not only used for the delivery of proteins and small chemical drugs but also cell delivery and tissue regeneration. At present, carrageenan is used as a polymer matrix in the form of hydrogels, wafers, suppositories, oral suspension, pellets, beads, suspensions, *etc.* However, based upon the excellent properties of carrageenan such as its gelling properties, viscosity, and strong negative charge, it is used for transdermal, oral, nasal, and ophthalmic release of therapeutic delivery. By increasing the knowledge of its properties, it is further used in other applications. Its different forms such as kappa, lambda, and iota, *etc.* due to their distinct properties enable them to be used for complex drug delivery systems. Furthermore, our current knowledge about their basic properties may provide a strong foundation for further use in advanced therapeutic delivery systems.

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Chapter 13

Xanthan Gum in Drug Carriers

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13.1 Introduction

13.1.1 History of Xanthan Gum

The fully biodegradable natural polymer *i.e.*, xanthan gum was first discovered in 1950 by Allene Jeans and their co-workers at Northern Regional Research Center (now known as National Research for Agricultural Utilization) of United States Department of Agriculture, Peoria, III (USDA). Xanthan gum was industrially produced in 1960 and then commercialized in 1964 by Kelco Company with the trade name “Kelzan”.¹ The gum was further approved in 1969 by United States Food and Drug Administration (USFDA) as a food additive for industrial and technological applications with no limitations of quantity.^{2,3} Afterwards, xanthan gum became the second non-toxic, industrial biopolymer after dextran to be commercially available in the market. The extensive research on xanthan gum was further carried out to evaluate its safety and toxicological properties in pharmaceutical and food applications. Additionally, the gum was also approved as an emulsifier and stabilizer in the food industry in the Code of Federal Regulations (CFR).⁴

13.1.2 Production

Xanthan gum is an anionic exopolysaccharide obtained by the aerobic fermentation of sugar by various species of *Xanthomonas* bacteria such as *X. citri*, *X. phaseoli*, *X. gummisudans*, *X. axonopodis*, *X. vascularium*, *X. arboricola*, and *X. fragaria*, while industrially, the gum is produced employing *X. campestris* NRRL B-1459.³ The industrial production of xanthan gum is estimated to be 30 000 ton per year through the batch process.^{5–7} Basically, a small quantity of preserved culture of *X. campestris* is grown on solid or in liquid substrates for two days at 28–30 °C to obtain the desired inoculum. The inoculum so obtained is transferred into large bioreactors where fermentation is allowed to proceed for at least 100 h. A number of factors such as pH, temperature, medium constituents, type of bioreactor, speed, operation mode, and oxygen concentration are reported to affect the production of this microbial gum.^{8–10}

After completion of the fermentation, the broth containing gum, bacterial cells, and other added chemicals is either centrifuged or filtered to recover the xanthan gum.¹¹ Finally, precipitation was done using water-miscible non-solvents (acetone, ethanol, and isopropanol) followed by pH

adjustment as shown in Figure 13.1. The gum has a high molecular weight ranging from 2×10^6 to 20×10^6 Da and voluminous (214.21 dl g^{-1} , η) based on variation in fermentation conditions and aggregation of multiple single chains.¹²

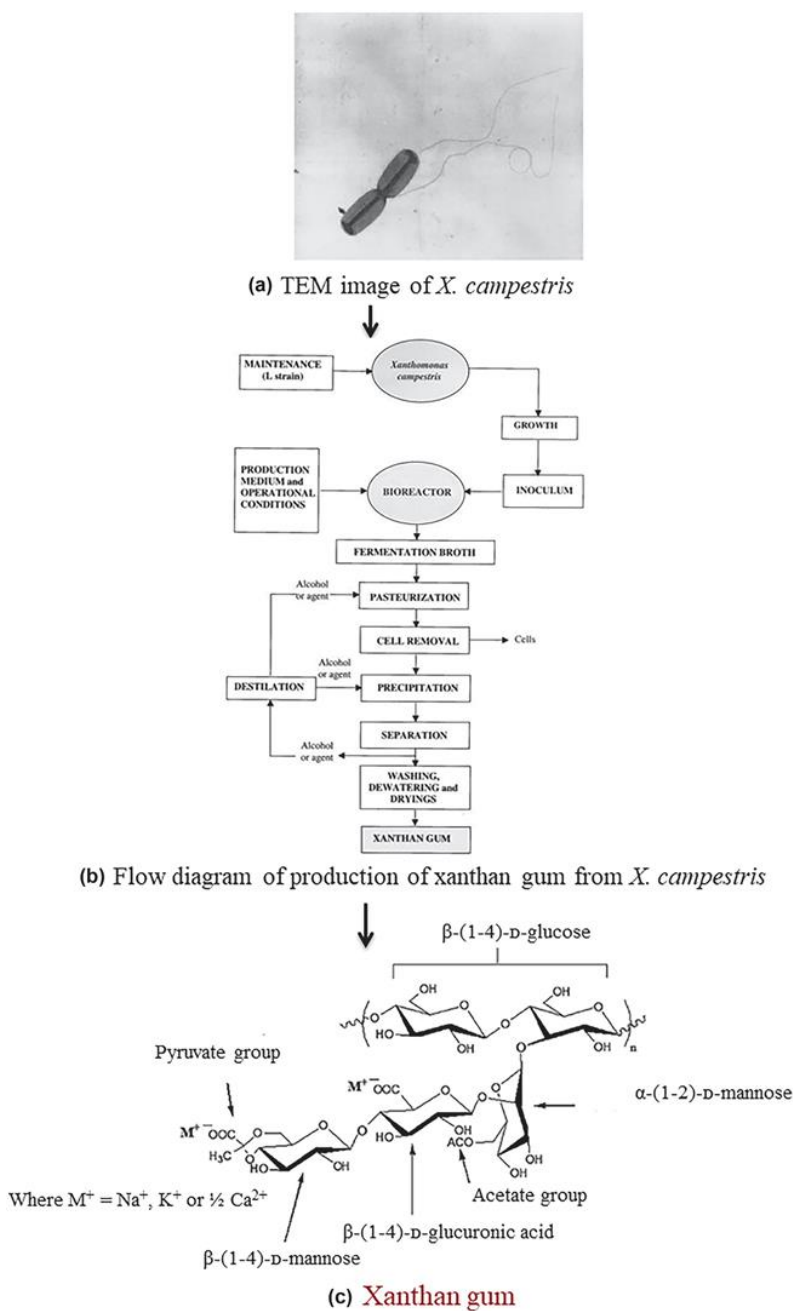


Figure 13.1 Xanthan gum and its production. (a and b) Adapted from ref. 13 with permission from Elsevier, Copyright 2000; (c) adapted from ref. 4 with permission from Elsevier, Copyright 2021.

13.1.3 Chemical Composition and Structure

Firstly in 1975, the primary structure of xanthan gum was established to consist of pentasaccharide subunits with branched polymeric chains. Xanthan gum contains α -glucosyl, α -mannosyl, and α -glucuronyl acid residues in 2.8 : 2.0 : 2.0 ratios with different proportions of *O*-acetyl and pyruvyl residues depending upon the polymer source.^{13,14} The primary structure of xanthan comprises a β - α -glucopyranosyl unit linked at the 1–4 position, while the secondary structure of xanthan gum is a 5-fold right-handed helical matrix, stabilized with a covalent bond with a diameter of 1.9 nm and pitch 4.7 nm.^{4,15} The side backbone of gum is a combination of β - α -mannose and α -glucuronic acid linked with β -(1–4). Trisaccharide side chain of xanthan gum contains a unit of α -glucuronic acid in between the two units of α -mannose linked at the O-3 position of every glucose unit. Because of the resemblance of rigid backbone structure of gum (β -1–4- α -glucose) with a cellulose structure, common cellulases and enzymes cannot attack the tri-saccharide side chain, making the xanthan gum more stable. With this, the thermal stability of xanthan gum was found optimum due to the ordered helical structure. Moreover, low salt concentration and high temperature stabilize the order conformation and therefore produces the highly stable xanthan gum which also ensures the presence of dimeric or double strands in the xanthan structure.^{16,17}

13.1.4 Physico-chemical Properties

The xanthan gum appears as a cream colored and odorless powder. It is stable under high temperature and over a wide pH range from acidic to alkaline and enzymatic conditions.³ The gum gets degraded completely within two days (DIN 38412-L25) because of its natural source. It also confronts the attack of common cellulases. Xanthan gum shows a proficient thickening ability with better solution viscosity at low concentration. However, at high temperature, gum solution exists as a disordered coil while co-operative conformational transition in H-bonding occurs due to the addition of salt or on cooling.^{17,18} Like other gums (except starch products), xanthan gum does not digest easily but decreases the food calories level and refines the food passage through the gastrointestinal tract.¹⁹ The calorific content of gum is almost 0.6 kcal g⁻¹. The gum is soluble in hot or cold water but requires intense agitation to prevent the formation of lumps. The gum solutions act like a non-Newtonian fluid and show high pseudoplastic behavior which promotes the pumpability, pourability, and mixability performance, with sensory qualities of food products.²⁰ However, with varying time or shear stress, its apparent viscosity and degree of aggregation is affected significantly which is directly related to the conformational status of polymeric chains.²¹ Dissolution, temperature, biopolymer concentration, and high shear rate also affects the viscosity of xanthan gum. It has high thermal stability against hydrolysis as compared to other polysaccharides due to its ordered helical formation which protects its depolymerization. Though pH or fermentation conditions do not influence the viscosity of xanthan gum solutions, the sterilization step somewhat affects it.¹⁴ Due to the excellent rheological properties and stability, xanthan gum is highly recommended for use as an immunological, suspending, thickening, and stabilizing agent.^{22,23}

13.2 Modifications

Xanthan gum has multi-functional and requisite properties which makes it one of the significant substances for various food and pharmaceutical applications. Besides its advantageous properties, some characteristics of xanthan gum like variation in viscosity, low surface area, probability of bacterial contamination, slow dissolution rate, batch to batch variation, and non-restricted hydrolysis limits its usage in industries. So, to overcome these drawbacks and improve the physical, chemical,

and even biological properties, modifications of xanthan gum have been accomplished by various researchers.

13.2.1 Carboxymethylation

The carboxymethylation reaction is a well-known modification technique which is extensively used worldwide, and which requires a couple of chemicals like monochloroacetic acid and sodium hydroxide, and is carried out in less time. The reaction is based on Williamson's synthesis reaction where the primary and/or secondary alcohol group of polysaccharides are etherified in two steps only.²⁴ The first step is the formation of an alkoxide group using sodium hydroxide followed by the attachment of a carboxymethyl group on the polymer backbone using monochloroacetic acid carried out at a certain temperature.²⁵ This modification technique was also applied to xanthan gum to improve the physical strength (elastic modulus), viscosity, hydrophilicity, and chemical reactivity of the polymer. Ahuja *et al.* modify the xanthan gum and synthesized its carboxymethyl derivative. Initially, xanthan gum was dispersed in an aqueous solution of sodium hydroxide (45% w/w), followed by the addition of monochloroacetic acid (75% w/w) with the aid of stirring at 70 °C for 30 min. Afterward comparison of native and modified xanthan gum revealed that carboxymethylation reaction decreased the particle size and viscosity of pure gum which eventually increases the rate of hydration and therefore faster matrix dissolution. Furthermore, the carboxymethyl derivative of xanthan gum was utilized to prepare a matrix tablet of diclofenac sodium for drug delivery applications.²⁶ Yahoum *et al.* synthesized the carboxymethyl xanthan derivatives by employing the etherification reaction with different ratios (2,4,6) of xanthan gum and monochloroacetic acid. The reaction was carried out by suspending xanthan gum (5 g) in sodium hydroxide (16 N), followed by the addition of a specified amount of monochloroacetic acid in the reaction mixture and this mixture was further heated at 50 °C for 4 h. Degree of substitutions (0.5–3) of different carboxymethyl derivatives were found proportional to the xanthan gum and chloroacetic acid ratios. It was observed that a decrease in molecular weight with an increase in the degree of substitution accompanied with a decrease in steric hindrance and easy accessibility of hydroxyl groups of carboxymethyl xanthan gum to water molecules which resulted in better solubility due to faster hydration. The study also revealed that after the etherification reaction, the crystallinity of carboxymethyl xanthan derivatives (CMXG4 and CMXG6) are increased to a partial level while native gum was amorphous in nature. However, no change was noticed in the shear thinning behavior of xanthan gum after carboxymethylation reaction but the viscoelastic properties were different, which may be due to the increase in ionic strength and decrease in viscosity. The transparency of xanthan gum gels also enhanced which enabled them to be suitable excipients for liquid (sugarless gargles) and semisolid (hydrogel) formulations.²⁷ Apart from this, a microwave irradiation method can also be employed to carry out the ultrafast (75 s) synthesis of carboxymethyl xanthan gum. The eco-friendly microwave method not only reduces the reaction time period but also improves the multiple properties of carboxymethyl derivatives.^{28,29} Furthermore, the produced carboxymethyl derivative of xanthan gum can be used for the synthesis of many pharmaceutical, cosmetics, and food formulations which are discussed below in detail.

13.2.2 Grafting

Graft polymerization, an effective and vulnerable technique has been used in recent years for modification of polysaccharides to obtain modified polymers with desired features. The presence of the various functional groups like carboxyl, hydroxyl, and amine on the main chain of polysaccharides provides suitability in grafting of other polymeric chains on the backbone of a

polysaccharide resulting in the emergence of new polymeric chains from different points of the backbone. Usually, vinyl monomers such as *N*-vinyl-2-pyrrolidone, acrylamide derivatives, and epoxide groups are employed for grafting reactions. The conventional chemical reactions are time consuming and result in the formation of a homopolymer due to the use of an initiator (e.g. ammonium persulfate, potassium persulfate) which is undesirable. These drawbacks can be overcome by using a microwave and UV-assisted method.³⁰ Kumar *et al.* synthesized the graft copolymer hybrid of xanthan gum and poly(*N*-vinyl-2-pyrrolidone) using a free radical polymerization technique in which peroxy monosulfate/thiourea was taken as the redox pair. The levofloxacin-loaded graft copolymer hybrid exhibited 80% drug release for 36 h in a controlled manner which justifies that grafting of natural polysaccharides is an acceptable technique for targeting specific and controlled drug delivery of highly soluble drugs.³¹ Acrylamide grafting is also a widely used technique carried out on various natural or synthetic polysaccharides. Graft copolymers of xanthan gum and acrylamide were synthesized by Deshmukh and Singh (1986) using a ceric-ion-initiated solution polymerization technique and were further analyzed for the biodegradation, drag reduction efficiency, and shear stability. It was found that a grafted compound has considerable enhancement in the drag reduction efficiency and biodegradation resistance property.³² Mundargi and their team synthesized the grafted xanthan gum using a free radical polymerization method by varying the concentrations of acrylamide (1 : 5, 1 : 10). Grafting of acrylamide on xanthan gum makes it a suitable controlled release matrix for antihypertensive drugs (atenolol and carvedilol).³³ Sand *et al.* performed the graft copolymerization of xanthan gum with 2-acrylamidoglycolic acid using bromate/thiourea as a redox pair and revealed that by grafting thermal stability and resistance to biodegradation, flocculation improved up to a considerable extent. The swelling behavior of grafted xanthan gum was also increased due to an increase in grafting percentage and the hydrophilic nature of 2-acrylamidoglycolic acid.³⁴ Another example of the grafting of acrylamide on xanthan gum involved the mechanism of the formation of macroradical at the alkoxy group of the polymeric backbone and then the occurrence of the reaction of acrylamide on the active site of the macroradicals. Various reaction conditions were optimized during the reaction which revealed that a longer reaction time decreases the intrinsic viscosity. Also, oxygen formation at a high concentration of potassium persulfate resulted in the decreased grafting percentage and chain degradation.³⁵ Ellela *et al.* (2017) synthesized biodegradable grafted xanthan gum by employing poly(vinyl imidazole). The grafting percentage of xanthan gum was increased with an improvement in thermal and antibacterial properties against *Escherichia coli* and *Staphylococcus aureus*. Furthermore, the addition of protein or genes can enhance the efficacy and properties of a synthesized formulation.³⁶ Ellela *et al.* (2021) carried out the encapsulation of bovine serum albumin (BSA) in grafted xanthan gum prepared using poly(*N*-vinyl imidazole). The formulation showed better efficacy as a pH sensitive carrier for protein delivery in the intestine. The electron micrographs gave the confirmation of diffusion of BSA in the porous polymer matrix. Further analysis of the swelling capacity and release rate of crosslinked grafts showed higher drug release at alkaline (pH 7.4) than acidic (pH 1.2) conditions following on the Fickian mechanism. Crosslinked grafts exhibited good inhibitory effect against two microorganisms – *Staphylococcus aureus* and *Aspergillus niger* confirming their potential for further use as antimicrobial protein carriers for protein delivery in the gastrointestinal tract.^{4,37}

Roy and co-workers grafted the hydrophobic octylamine on the carboxylic groups of xanthan gum using carbodiimide-mediated peptidic coupling reaction. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) and *N*-hydroxysuccinimide (NHS) reagents were used to activate the carboxylic groups and the reaction was carried out in an aqueous environment at room temperature. Native and modified xanthan gums were analyzed using fluorescence analysis and found that

fluorescence curves of both gums were almost similar with a difference in critical concentration which decreased with the increase in grafting density. In rheological studies, both gums showed the same shear-thinning behavior at high shear rate but at low shear rate native gum presented a Newtonian-plateau while the modified gum showed the same shear thinning behavior. There was no effect on the conformation of xanthan gum after modification with hydrophobic moieties.³⁸ Roy *et al.*, in 2016, investigated the shear viscoelasticity of native and hydrophobically-modified xanthan gum at the silicon oil/water interface. Xanthan gum is capable of adopting two confirmations, at low temperature a rigid confirmation and at high temperature a flexible one. The results exhibited that before thermal treatment both native and modified xanthan gum absorbed and created rigid helices at the silicon oil/water interface. At high temperature, highly modified xanthan gum chains are not affected much and adopt the same organization while the organization of less grafted xanthan gum was extremely different. The higher the grafting density means high hydrophobic moieties, and the higher the conformation temperature and decrease in the flexible fragments that can be absorbed on the oil/water interface. With these interesting interfacial properties, hydrophobically-modified xanthan gum can be used as a stabilizing agent in emulsions.³⁹

13.2.3 Phosphorylation

Phosphorylation is another method of modification of many natural and synthetic polymers. It involves the addition of phosphate ions on the backbone of polysaccharides. Phosphorylation of xanthan gum has also been carried out by some researchers but not much explored. Leone and team investigated the complex of silver ions and phosphorylated xanthan gum as a viscosity enhancer with antibacterial properties. The xanthan gum was phosphorylated using sodium trimetaphosphate as the phosphorylating agent and then silver ions were incorporated in the phosphorylated xanthan gum. The prepared complex was used to prepare eye drop formulations which exhibited pH, osmolality value, viscosity, and transparency close to human tears. Furthermore, they also observed that the eye drop formulation was able to suppress bacterial proliferation without any cytotoxic effect.⁴⁰ Figure 13.2 shows a different derivative of xanthan gum after the modification process.

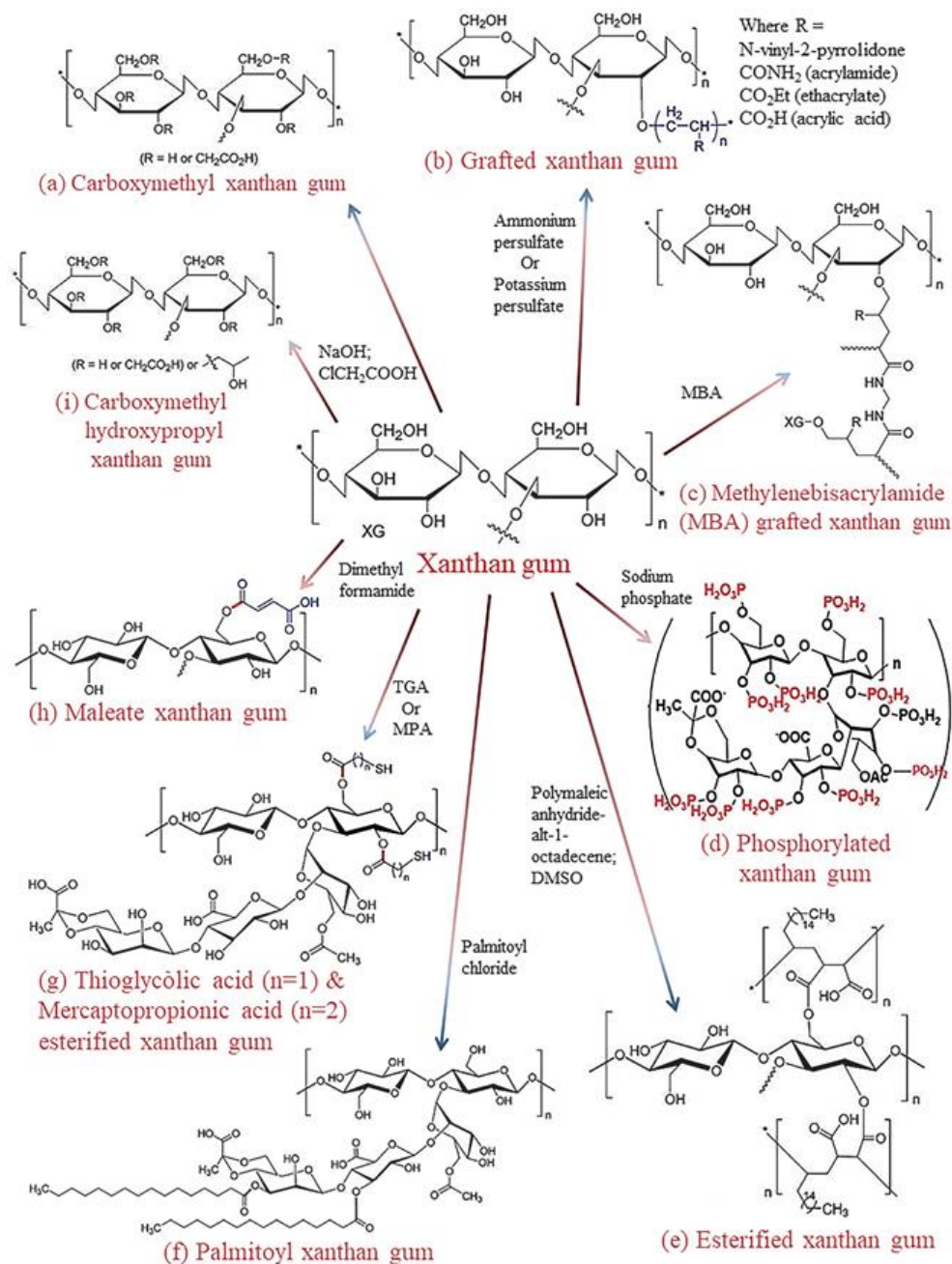


Figure 13.2 Modifications of xanthan gum. (a, e and f–i) Reproduced from ref. 85, <https://doi.org/10.1039/D0RA04366D>, under the terms of the CC BY 3.0 license <https://creativecommons.org/licenses/by/3.0/>; (b and c) adapted from ref. 30 with permission from Elsevier, Copyright 2021; (d) adapted from ref. 3 with permission from Elsevier, Copyright 2019.

13.2.4 Esterification

The esterification process is one of the widely used modification techniques which involves the addition of ester groups on the main chain of the polymer by reaction with unsaturated acid compounds such as acrylic acid, acryloyl chloride, and maleic anhydride *etc.* Esterification of xanthan gum with an acrylic acid has been carried out by researchers to develop a biodegradable 3D hydrogel network or matrix for drug delivery applications. Hamcerencu *et al.* (2007), synthesized the

acrylate and maleate esters of xanthan gum employing *N*'-[3-(dimethylaminopropyl)]-*N*-ethylcarbodiimide hydrochloride (DEC) as an activator. The research study revealed that maleate esters were more reactive than acrylate esters and suitable for controlled drug release formulations.⁴¹ Anghel *et al.* employed two different methods to perform the esterification of xanthan gum. In the first method, dicyclohexylcarbodiimide (DCC) was used as the coupling agent and in the second method, acrylic acid was used. The authors adopted the second method due to difficulty in the removal of the by-product formed in the first method. During the process, the double bond formed in the structure caused establishment of strong hydrophobic interactions with improvement in mechanical strength like flexibility and resilience. Amoxicillin and ketoprofen were also encapsulated in the polymer matrix and it was found that drug-loaded cross-linked phosphorylated xanthan gum showed controlled release followed by a non-Fickian mechanism. Esterified xanthan gum also showed inhibitory action against both Gram-positive and Gram-negative bacteria which confirm that this matrix can be used as a bioactive carrier in various pharmaceutical and cosmetic applications.⁴²

An esterification technique has also been used as chemical modification of xanthan gum for the aqueous dispersion of double-walled carbon nanotubes. Three types of esters of xanthan gum were synthesized using 2,3-diphenylmaleic anhydride, phthalic anhydride, and *N,N*-dimethylformamide. It was observed that at acidic pH 5, all derivatives were found to form stable suspensions due to the hydrophobic character produced by non-covalent linkages.⁴³ Thiol derivatives of xanthan gum were synthesized using an esterification method by using mercaptopropionic acid and thioglycolic as esterifying agents. By the esterification method, the mucoadhesive property of xanthan was also enhanced due to the disulfide bond formation between mucus and thiol derivatives. Metronidazole impregnated thiolated xanthan gum showed drug release in a sustained manner for a prolonged period.⁴⁴ Using succinic anhydride, esterification of xanthan gum can also be performed and its hydrogels can be prepared with applications in ionic strength-sensitive drug release formulations. Dimethylaminopyridine (DMAP) was used as a catalyst in the reaction. After successful succinylation of xanthan gum, enhancement in gel performance and elasticity was observed. The synthesized drug-loaded hydrogels exhibited an antimicrobial effect against *Staphylococcus aureus* and also non-toxicity toward human lens epithelial cells (HLEC).⁴⁵

13.2.5 Miscellaneous Modifications

Apart from the above-mentioned derivatizations, xanthan gum has also been modified by techniques like mechanical modification, oxidation, acetylation, and deacetylation *etc.* Eren *et al.* (2015), carried out the mechanical modification of xanthan gum by treating it with high pressure homogenization and found the gradual loss in structural network with an increase in intensity of the treatment which was proved by the decrease in viscosity and viscoelasticity properties and increase in hydrodynamic volume of modified xanthan gum. With mechanical modification, the molecular weight of gum was decreased and polydispersity was increased, due to which a decrease in rheological functions has been observed.⁴⁶ Synthesis of oxidized xanthan gum by using various aldehyde compounds employing a periodate oxidization method has been carried out by the researchers for the use of polymer as a crosslinking agent. Guo *et al.*, prepared gelatin edible films using the native gum and oxidized gum and found a decrease in crystallinity upon oxidation. Oxidation of xanthan gum enhanced the water resistant properties,⁴⁷ and thermal and mechanical strength of gelatin edible films which was due to the covalent linkage between the polymers. Ultraviolet barrier properties were also improved due to the introduction of aldehyde groups during oxidation of the gum.⁴⁸

Acetylation is other modification technique which involves the addition of acetate groups on the backbone of the polysaccharide by using different chemicals *e.g.*, acetic anhydride. The objective of the modification was to improve the polymer properties to form a thermoplastic material for its further applications. Muljana *et al.* (2018), synthesized acetylated xanthan in densified CO₂ at different pressures and observed morphological changes involving the enhancement in agglomeration of xanthan gum particles indicating the hydrophobicity due to the reaction. Acetylated xanthan gum was thermally more stable than native xanthan gum which may be due to the increase in the molecular interactions.⁴⁹ Deacetylation of xanthan gum is also a modification process which includes the removal of acetyl groups present on mannose residues of the side chains of the polymer backbone. Tako and Nakamura in 1984 investigated the rheological properties of deacetylated xanthan gum in aqueous solution and found that after deacetylation, the apparent viscosity decreased with temperature for low concentration solution but increased with high temperature for high concentration solution while the dynamic viscoelasticity of various concentration solutions decreased with an increase in temperature.⁵⁰ The above results also suggested that acetyl groups present on the mannose residues of polymer side chains have a stabilizing effect due to their intramolecular connections and this may be the reason for the greater flexibility in the side chains of xanthan gum after deacetylation.⁵¹ In 2007, Khouryieh *et al.* studied the effects of deacetylation of xanthan gum on the rheological functions of xanthan–guar interactions and their observations suggested that due to the greater flexibility in the modified xanthan gum as already mentioned in the above research studies, the intrinsic viscosity and elasticity of the modified complex were higher than the native xanthan gum and guar gum complex.⁵² The combination of deacetylated xanthan gum derivatives and chitosan can be used as an excipient in sustained release dosage forms in pharmaceutical industries. Boudoukhani *et al.* prepared tramadol matrix tablets by using the chitosan–deacetylated xanthan gum derivatives complex as the tablet matrix and observed that with a high degree of deacetylation, the drug release rate decreased and proved to be the best candidate for prolonged drug delivery.⁵³

13.3 Xanthan Gum for Drug Delivery Applications

Natural origin polymers draw special attention in almost every field because of their versatile properties like sustainability, biocompatibility, biodegradability, easy availability, non-toxicity, and cost-effectiveness.⁵⁴ Among these, xanthan gum is one of the suggested and prominent natural exopolysaccharides which has been extensively investigated for drug delivery and tissue engineering applications. Many formulations of xanthan gum have already been explored in different areas of the pharmaceutical field which are explained below in detail.

13.3.1 Nanoparticles

Nanoparticles, as the name indicates, are small-sized particles ranging from 1–100 nm. Polymeric nanoparticles are a favorable formulation because of (a) providing stability to labile molecules, (b) controlled delivery of drug at target sites, (c) improved bioavailability, and (d) better binding ability to the surface. Considering all these advantages, xanthan gum nanoparticles were also prepared by various researchers using metals, colloids, and other polymers.⁵⁵ Pooja *et al.* prepared the environment-friendly gold nanoparticles by a chemical reduction method using xanthan gum as the reducing agent. The effect of the concentration of gum, gold, reaction temperature, and time was evaluated. The optimum concentrations of gum and gold were found to be 1.5 mg ml⁻¹ and 10 mM respectively, with the reaction at 80 °C for 2 h. The xanthan-stabilized gold nanoparticles were

loaded with doxorubicin *via* incubation at room temperature overnight with an entrapment efficiency of approximately 71.4%. The prepared doxorubicin-loaded gold nanoparticles of xanthan gum show aspherical shape with a mean hydrodynamic diameter (41.2 nm), polydispersity index (0.35), and surface zeta potential (– 47.2 mV). The decrease in zeta potential (–29.1 mV) was observed after drug-loading in nanoparticles due to interaction of the negatively-charged acidic group of xanthan gum and positively-charged amine group of doxorubicin. On evaluation for *in vitro* release, 98% of the drug was observed to release from gold nanoparticles in sodium acetate buffer (pH 7.4) while 83% of the drugs were released in phosphate buffer solution (pH 4.5). The increase in % release in sodium acetate buffer was attributed to the protonation of the amine group of doxorubicin which enhances its solubility and hydrophilicity at low pH. Other than this, xanthan gum-based gold nanoparticles were found to be non-toxic and biocompatible in a hemolysis study with better cytotoxicity toward lung cancer cells.⁵⁶

Alle *et al.* utilized anionic carboxymethyl xanthan gum as capping, reducing, and stabilizing agents. The researchers formulated the gold nanoparticles of doxorubicin using a microwave-assisted synthesis method. In the research work, an aqueous stock solution of carboxymethyl xanthan gum (2% w/v) was mixed with gold chloride (1 mM) and irradiated under a microwave power of 800 W and temperature 104 °C for a time period of 15–90 s. The obtained wine-red gold nanoparticles were centrifuged and redispersed in water to obtain a final concentration of 0.1 mg ml^{–1}. Then the desired amount of doxorubicin drug was loaded in the prepared nanoparticles with the help of stirring and incubating, followed by centrifugation to obtain drug-loaded polymeric nanoparticles. However, three different methods were adopted *i.e.* autoclave, sonication, and microwave-assisted to synthesize the gold nanoparticles but amidst those, the microwave synthesis method was found best to prepare the colloidally stable and uniform small-sized nanoparticles. It was observed that a greater concentration of carboxymethyl xanthan gum (1.0%) increases the zeta potential and decreases the particle size of the formulation which can be ascribed to the effective capping of carboxymethyl xanthan gum on gold nanoparticles. However, a precursor concentration (gold chloride) did not show any considerable effect except for increasing the surface plasmon resonance and particle size. Doxorubicin drug was almost completely entrapped in the nanoparticles with entrapment of 96%. The zeta potential of spherical shaped gold nanoparticles was found to be –49.3 mV which was decreased to –28.32 mV after drug loading. It was also noticed that the release of drug from the developed nanoparticles varies at different pH. In a tumor tissue environment at acidic pH 5.3 and 6.6, 98% and 89% of the drug were released while in phosphate buffer (pH 7.4), insignificant release (6%) of doxorubicin drug was shown even after 12 h of incubation. However various studies recommended that pH-sensitive formulation of doxorubicin drug can be employed for cancer therapy which is attributed to the involvement of the endocytosis process in the internalization of nanocarrier into tumor cells at acidic pH 5.3 and 6.6.²⁹ After analyzing the drug release behavior at different pH, cytotoxicity efficacy was also confirmed. Table 13.1 enlists the many other metallic or polymeric nanoparticles and microparticles of xanthan gum or its derivatives with their different applications.

Table 13.1 Xanthan gum-based nanoparticulate and microparticulate formulation for drug delivery applications.

Composites	Method of preparation	Properties	Advantages/applications	Ref.
Xanthan gum stabilized curcumin loaded PEGylated gold nanoparticles	Hydrogen tetrachloroaurate(III) hydrate was used to prepare gold	A colloidal stable deep yellow colored hydrophilic	– Non-toxic – Enhanced cytotoxicity	63

	nanoparticles of xanthan gum and then PEG was incubated followed by curcumin.	solution was found of nanosize (size: 95 ± 2 nm; PDI: 0.1 ± 0.01 ; zeta: -28.1 ± 1.1 mV). Also sustained drug release rate (50% in 4 h) was found in pH 7.4	– Biocompatible and improved pharmacokinetics – Target drug delivery for cancer treatment
Tolbutamide loaded xanthan gum stabilized green gold nanoparticles	Gold solution was added in pure xanthan gum solution and then stirred magnetically for 2 h at 65 °C. Tolbutamide was loaded in nanoparticle dispersion.	Purple colored gold nanoparticles were formed having the particle size (168.33 ± 4.06 nm), zeta potential (20.43 ± 0.76 mV) and an effective drug loading capacity ($76.39 \pm 3.27\%$).	– Enhanced the potential of insulin secretion.
Bergenin loaded xanthan gum stabilized silver nanoparticles	Solution of xanthan gum and silver nitrate was mixed in 1 : 1 and stirred homogenously. Then the dark yellow green synthesized silver nanoparticles were added in bergenin solution	The particle size, PDI, and zeta potential of drug loaded metallic nanoparticles was found to be 189.20 ± 2.09 nm, 0.41 ± 0.01 and -16.2 ± 0.40 mV respectively. Almost 87% bergenin was successfully loaded in the prepared nanoparticles.	– Stable against plasma proteins – Non-toxic – Anti-inflammatory and anti-arthritis efficiency
Nanoscale zero-valent iron (NZVI)–xanthan dispersion	Colloidal dispersion of nanoscale zero-valent iron slurries was added in water	Negative zeta potential value because the pH is higher than	– Xanthan polymer trapped the colloidal particles and hence

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	with ultrasonication and then xanthan gel was dosed in recirculating suspension.	the isoelectric point.	stabilized the dispersion. – Decrease in aggregation viscosity.	
Xanthan gum-coated iron nanoparticles	Bacterial cells were grown in brain–heart infusion broth and then transferred in ferric citrate as an iron precursor and incubated at 30 °C.	Iron oxide nanoparticles were oval, spherical, and polymorphous as shown by TEM. Diameter of the nanoparticles was in the size range of 20–80 nm	– Biologic and sustainable method was developed to form xanthan-coated iron nanoparticles.	67
Xanthan gum and lysozyme nanoparticles	Both xanthan gum and a lysozyme solution of concentration 1 mg ml ⁻¹ were prepared by stirring at 6 h and 2 h respectively, followed by adjusting its pH to 11.8. Variation in weight ratios (3 : 1, 2 : 1, 1 : 1, 1 : 2, 1 : 3), heating time (5–60 min), and incubation temperature (40–90 °C) was analyzed during formulation.	After investigating the experimental conditions, the final selected parameters were found to be weight (1 : 1), incubation temperature (80 °C), and heating time (15 min) to prepare spherical size-controlled nanoparticles (61.7–108 nm).	– Stable over one month – More hydrophobic in nature – Useful for protein/polysaccharide nanoparticle formulation	68–70
Xanthan gum functionalized SPAN nanoparticles	Nanoparticles of xanthan gum-based sorbitan monooleate (SPAN 80) were prepared using oleylamine and ethanol:water solution. Enhanced	Average size of pEGFP-loaded SPAN nanoparticles of 153.5 ± 17.8 nm, PDI of 0.102 ± 0.036, and zeta	– Stable up to 12 months at room temperature and 37 °C – <i>In vivo</i> plasmid DNA delivery to the vascular endothelium of organs	71

	green fluorescent protein plasmid (pEGFP) were then encapsulated, followed by lyophilization using cryoprotectant.	potential of -40.6 ± 4.6 mV were found	– Promising gene delivery to specific targets for treatment of vascular related disorders	
Xanthan-based polysaccharide/protein nanoparticles	Nanoparticles of xanthan gum, BSA, and curcumin were prepared by applying a temperature treatment protocol	Thermally stable nanoparticles represent characteristic ratios (R_g/R_h) of about 1.1–1.3, while the zeta potential was calculated to be -45 mV at pH 7. The formulation was found stable with or without curcumin for up to one week at room temperature.	– Suitable as a pharmaceutical and nutrition product for biomedical or food delivery application	72
Xanthan gum solid lipid microparticles (SLM) with added whey protein isolate and curcumin emulsion-filled gels	SLM of xanthan gum loaded with curcumin were prepared using surfactant (tween 60 and span 80) and lipid phase (tristearin and babacu oil). An emulsion gel of whey protein isolate and xanthan gum was synthesized using a homogenizer in which solid lipid microparticles were added. Then the effect of different concentrations of	Low concentration of xanthan gum shows a synergistic effect between protein and polysaccharide and produces a more rigid structure while more gum produces aggregates. While a high concentration of SLM increases the stiffness and stress of rupture which ultimately	– Rheologically and mechanically stable – Effective curcumin encapsulation and delivery – Promising alternative for healthy food production	73

	gum and solid lipid microparticles were observed.	improve the mechanical and rheological properties.	
Methacrylic monomer-based xanthan gum microparticles	Aqueous suspension polymerization of methacrylate, dimethacrylate, and xanthan gum was prepared in a cylindrical reactor and then their microparticles were formed using a free radical initiator (ammonium persulfate and benzoyl peroxide)	Particle size and % yield of copolymerization of all prepared microparticles were found in the range of 160–316 nm and 85–98% respectively.	74, 75 – High surface area and better sorption capacity – More precise for retention, delivery, and controlled release of various drugs
Chitosan-xanthan-quercetin microparticle added oral tablets	Microparticle powders of chitosan (0.65% w/v), xanthan gum (0.65% w/v), and quercetin (0.1 g) were prepared using a spray dryer. Then mixture of microparticle powder and lactose (1 : 4) were directly compressed into the form of tablets, on which Eudragit® L125 was coated.	Average particle size and zeta potential of prepared microparticles were found to be $5.3 \pm 2 \mu\text{m}$ and $32 \pm 6 \text{ mV}$. The encapsulation efficiency of quercetin was noted to be $63 \pm 7\%$ and the % yield of microparticles was $57 \pm 3\%$. This lower % yield and encapsulation efficiency were due to sample loss during preparation in the spray dryer. Approximately 94% of drug was released	76, 77 – Easy reproducible and cost-effective oral dosage form – Target specific and sustain delivery of quercetin – Colon specific drug delivery for the treatment of inflammatory based disorder

Celecoxib-loaded xanthan gum and locust bean gum-based biodegradable microparticles	Emulsification method was utilized to prepare the microparticles where the aqueous phase (xanthan, locust bean gum, and celecoxib dispersion) and lipid phase (tween 80 in liquid paraffin) were mixed. To evaluate the better drug loading capacity, encapsulation efficiency, and % yield, central composite factorial design was also applied.	after 24 h, followed by the Korsmeyer–Peppas model. High concentration of drug/locust bean gum (% w/w) and drug:locust bean gum/xanthan gum (% w/w) increases the encapsulation efficiency (57.93–95.99%) and drug loading capacity (18.90–37.30%). However a lower level of drug/locust bean gum and high level of drug:locust bean gum/xanthan gum increases the %yield (84.12–91.28%).	78 – Stable at 25 °C/60%RH, 30 °C/75%RH, and 40 °C/75%RH for 180 days – Selected batch shows 91.8% release in a sustained manner for 24 h, followed by the Korsemeyer–Peppas model.
Xanthan gum and guar gum-added curcumin microspheres	Using an ionic cross-linking technique, microspheres of hydrophilic polymer–drug–alginate were prepared using a cross-linking agent (calcium chloride). Both xanthan and guar gum were used separately to prepare their respective microspheres.	Average particle size of all microspheres was observed in the range of 33.10–35.43 µm. Also, entrapment of the drug was more in xanthan microspheres than guar microspheres. A greater polymer concentration increases the	79 – Drug release at target site – Colon drug delivery applications

		particle size which ultimately leads to more surface area and sustained release behavior.	
Lysozyme-absorbed calcium carbonate and xanthan gum microspheres	Biomimetic mineralization process was considered to prepare mineralized microspheres. Mainly calcium carbonate (2 mg ml ⁻¹) was added in aqueous xanthan gum solution (1 mg ml ⁻¹) with continuous stirring, followed by the addition of sodium carbonate. Then the freeze-dried microspheres (10 mg) were added in lysozyme solution (10 ml) at pH 7 and 9 for complete absorption.	Spherical microspheres with diameter 0.3 μm, zeta potential -35.1 ± 0.98 mV, and surface area 21.67 ± 1.2 m ² g ⁻¹ were found to be thermally stable in the temperature range of 80–600 °C. The absorption capacity of lysozyme was twice that at pH 9 than at pH 7. Fluorescence intensity was also increased at pH 9.	80, 81 – Excellent adsorption properties – Xanthan gum acts as a morphology control agent for the mineralization of calcium carbonate
Interpenetrating polymer network (IPN) microspheres of verapamil, poly(vinyl alcohol), and xanthan gum	IPN microspheres were prepared using a water-in-oil emulsion crosslinking method. In the aqueous phase, both polymers were dissolved, followed by the addition of verapamil drug with continuous	The amorphous nature of verapamil-loaded microspheres was found to be thermally stable. However, the release effect after a change in polymer, drug, and cross-linking agent	82 – Act as suitable sustained release formulation for drug delivery applications

stirring. The obtained dispersion was added in the oily phase containing liquid paraffin oil, tween 80, and glutaraldehyde.	concentration was also noticed which revealed that almost all the drug was released within 10 h in all cases.
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Apart from the gold nanoparticles, the metal nanoparticles of silver, palladium, iron, and zinc using xanthan gum as capping and reducing agents were also prepared. To synthesize the silver nanoparticles, a mixture of xanthan gum (0.1% w/v) and silver nitrate solution was incubated for different time periods at different temperatures. The dark brownish yellow color of the formulation confirms the formation of silver nanoparticles. It was observed that temperature (100 °C), silver nitrate concentration (3 mM), and heating time (24 h) produce the thermally stable and spherically-shaped nanosized (5–40 nm) silver particles stabilized by xanthan gum. Besides this, its antibacterial and catalytic activity was also evaluated which makes it a reliable material for the development of pharmaceutical products.⁵⁷ Apart from gold and silver nanoparticles, palladium nanoparticles were also prepared using a hydrothermal method in an autoclave. A certain amount of palladium chloride and hydrochloric acid were dissolved fully to produce an aqueous solution of H_2PdCl_4 . Then xanthan gum solution was added to the prepared H_2PdCl_4 solution and kept in an autoclave at a temperature of 120 °C and pressure of 15 psi for 10 min. The final palladium nanoparticles were found to be black in color from what was an initially yellow-colored mixture. The spherically-shaped palladium nanoparticles of approximately 10 nm in size were evenly distributed in the polymer matrix. It was also found that the xanthan gum-stabilized palladium nanoparticles have good catalytic activity and pseudo first-order kinetics against 4-nitrophenol which can be further utilized for pharmaceutical applications.⁵⁸

Iron nanoparticles of various natural and synthetic polymers are also attracting various researcher's attention due to their advantageous properties such as eco-friendly nature, narrow distribution, large surface area, and small size.⁵⁹ Considering all these advantages, currently iron oxide nanoparticles of xanthan gum are being formulated and evaluated further. Among a number of synthesis methods such as chemical, solvothermal, microemulsion, hydrothermal, and sonochemical methods, a one step sonochemical method was adopted to produce iron oxide nanoparticles based on konjac and xanthan gum at room temperature. Basically, a certain amount of aqueous solution of konjac gum and xanthan gum (KG/XG) were mixed and then silica was added in the mixture to prepare a good blend. On the other hand, a solution of iron(III) chloride hexahydrate and sodium borohydride was prepared in ethanol and distilled water respectively. Afterward both solutions *i.e.*, the KG/XG gum solution and iron composite solution were mixed together and sonicated at room temperature for 30 min at different amplitude frequencies. The prepared solution was filtered through a syringe filter to obtain the final iron nanoparticles of two polymers and characterized further. However, optimization was carried out using dilute solution methodology to evaluate the intrinsic viscosity of the nanoformulation. Three different experimental parameters were chosen to prepare the nanoparticles such as sonication time (10–60 min), KG/XG blend mass ratio (1 : 1, 1 : 2, 1 : 4) and amplitude frequency (5–40%). The experimental study revealed that mainly the amplitude frequency affects the value of intrinsic viscosity as the frequency decreases, and the intrinsic viscosity increases while the smaller amount of sonication time and minimum blend mass ratio of KG/XG produces highly viscous nanoparticles. The particle size, polydispersity index, and zeta potential of the iron oxide nanoparticles as determined by the dynamic light scattering method were found to be 10 nm, 0.2 and

–36.7 mV respectively. On the basis of electron micrographs and the thermal stability of metal oxide nanoparticles the authors disclosed the uniform agglomeration of spherical nanoparticles on the polymer matrix with better thermal and aqueous stability.⁶⁰

Zinc oxide nanoparticles are other potent carriers to deliver drugs, cells, genes, and proteins to the target site of the human body. Its numerous advantages like broad spectrum antimicrobial activity, non-toxicity, reliability, biocompatibility, enhanced pharmacokinetics, and targeted delivery make it a suitable choice for multiple drug delivery applications.⁶¹ Several biopolymers are already being used in combination with metal oxide nanoparticles to enhance their efficacy and potency. Xanthan gum is also used in combination with zinc oxide nanoparticles for biomedical and drug delivery applications. Karakus *et al.* (2021), prepared metamizole sodium-loaded xanthan gum-based zinc oxide nanoparticles *via* an ultrasound-assisted synthesis method. Basically, an aqueous solution of xanthan gum (0.04 g in 50 ml) was prepared at 25 °C and kept in an ultrasonic bath. Simultaneously, a mixture of alkaline sodium hydroxide solution (10 ml, 0.002 M) with zinc nitrate hexahydrate (10 ml, 0.001 M) was added in an ultrasonic bath for appropriate homogenization and conversion to zinc oxide. Thereafter, metamizole drug solution (10 ml, 0.04 M) was dispersed in polymeric solution and stirred continuously for 10 min. Then the prepared drug-added metallic nanoparticles were dried at 50 °C for 24 h and stored at room temperature for further analysis. The formulated nanodrug shows high crystallinity with a crystallite size of about 35 nm. The nanoparticles were uniformly dispersed on a polymeric matrix with an average diameter of 80 nm. In addition, the drug release profile was also evaluated in pH 1.2 and 7.4 at 37 °C. It was observed that 65% of the drug had sustained released at pH 7.2 in 24 h while only 42% of the drug was released at pH 1.2. This increase in drug release percentage in basic medium was due to ionic interaction of the carboxylic acid group and negative charge of the nanocarrier.⁶²

13.3.2 Microparticles

Microparticles, as the name indicates, are particles of micrometric size ranging from 1–1000 µm. Microparticles may be of a matrix system (microspheres) or coated system (microcapsules). They have many significant drug delivery applications which include (a) control drug release rate, (b) effective encapsulation of drug, and (c) easy administration. Since 1989, several market formulations have been available in the market such as Lupron[®] Depot, Trenantone[®], Nutropin[®] Depot, Zoladex[®], Posilac[®], and Risperdal Cnosta[®] for the treatment of prostate cancer, growth hormone deficiency, and Schizophrenia diseases.⁸³ Various natural polysaccharides are being utilized to design microparticles because of the advantages such as better bioavailability, biocompatibility, and easy availability.

Xanthan gum and their derivatives have been utilized to produce microparticles for drug delivery, tissue engineering, regenerative medicine, and other biomedical applications.^{84,85} This section explains the use of xanthan gum or its derivatives in combination with other natural or synthetic polymers for microparticulate drug delivery applications. Banerjee *et al.* (2013) prepared the aspirin-loaded xanthan gum facilitated ethyl cellulose microparticles using multiple-emulsion solvent evaporation technology for intestinal specific target drug delivery. Firstly, an aqueous solution of xanthan gum and aspirin dissolved in acetone was mixed for complete dispersion. Then this dispersion was slowly added in ethyl cellulose and Span 80 solution under the aid of stirring. The obtained water-in-oil (w/v) emulsion was mixed in aqueous Tween 80 solution (0.5% w/v), and stirred appropriately for 3 h to evaporate excess solvent which led to the formation of microparticles. The prepared microparticles were filtered and washed with distilled water followed by drying in an oven overnight at 40 °C for further usage. However, variation in the concentrations of ethyl cellulose

(1% w/v and 2% w/v), Span 80 (0.05% w/v and 0.1% w/v), and the internal (5 ml and 10 ml) and external aqueous phase volume of xanthan gum (50 ml and 100 ml) was carried out to acquire stable, desired, and controlled release microparticles. It was observed that an increase in the concentration of ethyl cellulose from 1 to 2% w/v led to an increase in % yield from 80% to 92% and % drug entrapment efficiency from 16 to 49%. A faster release of aspirin was observed from microparticles prepared using ethyl cellulose at a concentration of 1% w/v as compared to 2% w/v. Similarly, a high concentration of Span 80 increased the % yield from 81 to 88, and % entrapment efficiency from 17 to 26%. Moreover, 10 ml of internal xanthan gum solution was preferred instead of 5 ml because of a high amount of internal phase volume of natural polymer which can be due to the small size of formed microparticles. It was also noticed that a change in the internal aqueous phase volume of xanthan gum decreases the % yield and entrapment efficiency of aspirin but did not influence the % release in acidic and alkaline buffer media. The effect of the external aqueous phase volume of xanthan gum was observed which revealed that the high volume increases the % yield and entrapment efficiency with a slight decrease in the particle size of microparticles, while a low amount of external phase volume shows faster release in pH 7.4 which follows the Korsmeyer–Peppas semi-empirical model. This prolonged release behavior of xanthan gum facilitated ethyl cellulose microparticles makes it prominent for gastric drug delivery systems.⁸⁶

Another study of solid lipid microparticles of xanthan gum in combination with whey protein isolate and curcumin was also carried out and is explained in Table 13.1.⁷³ Not only pure xanthan gum was used to prepare nanoparticles or microparticles formulation, but its modified derivatives were also utilized highly to improve the efficiency and capability of the formulation. Carboxymethyl xanthan gum is one of the highly recommended modified derivatives of xanthan gum which is used for formulating nanoparticles, microparticles, hydrogels, tablets, and beads. Maiti *et al.* (2007), prepared bovine serum albumin-loaded ionically gelled microparticles of carboxymethyl xanthan gum using Al^{3+} ions. The study investigated the influence of concentrations of carboxymethyl xanthan and bovine serum albumin, pH, and gelation time on the entrapment and release characteristics of bovine serum albumin. Briefly, the aqueous solutions of carboxymethyl xanthan gum (1–2% w/v) containing bovine serum albumin (30–50% w/w of polymer) with a pH of 4–7 were slowly added in aluminum chloride hexahydrate solution (1% w/v) drop by drop using a needle and allowed to gel for a time ranging from 5–60 min. The study reported that below the isoelectric point pH of 4.7 of bovine serum albumin, a lump was obtained instead of microparticles due to the interaction between the protein and the gum, so the microparticles were prepared at pH 6 and 7. Furthermore, it was observed that increasing the concentration of gum led to an increase in % entrapment and decrease in the release rate, while increasing the concentration of protein led to an increase in size, decreased entrapment, and faster release. On the other hand, the increase in gelation time resulted in a decrease in % entrapment, size, and the release rate. The results showed that the release of bovine serum albumin in acidic medium was similar to alkaline medium. The study concluded that if the release of protein in acidic medium is reduced by manipulating the formulation variables or by further modification of carboxymethyl xanthan, then carboxymethyl xanthan can be considered for oral sustained drug delivery.⁸⁷

13.3.3 Hydrogels

Hydrogels can be defined as biodegradable hydrophilic polymers consisting of three-dimensional cross-linked networks with the ability of water absorption a thousand times greater than their dry weight without dissolving in it while adopting a stable structure.^{88,89} The susceptibility of hydrogels to change with pH, temperature, and ionic strength enhances its applications in targeted drug delivery systems, biosensors, pharmaceutical, and biomedical fields. Biocompatible hydrogels with a good

water absorption and retention ability can be effective wound dressing agents. The wound healing rate in the presence of a hydrogel as the dressing material is enhanced because of quick absorption of exudates and the wet environment provided by the hydrogel; with no damage on the regenerated surface during the change of dressing and lack of bacterial dispersion at the wound surface.⁹⁰ The fabrication of hydrogels involves the combination of two biodegradable, biocompatible, and chemically modifiable polymers to tailor their existing properties and to generate some novel properties.⁹¹ Recently, the strategies of hydrogels synthesis by incorporating clays or nanoparticles have come into consideration with the improvement in mechanical, optical, and swelling properties of hydrogels.⁹² The concept of 'self-healing hydrogels' is very interesting, which is about the hydrogels that have the potential to deal with destructive factors and have the capability to regain their mechanical and rheological strength.

On the basis of their fabrication these hydrogels can be intrinsic (chemical bonds inside the polymer with the ability to restore the structure after some distortion) and extrinsic (healing agents incorporated inside the matrix) in nature.⁹³ Some researchers also developed injectable polymer–liposome hydrogels with physical, chemical, and biological stimulus with fabulous self-healing properties. Natural polysaccharides such as chitosan, xanthan gum, alginate, agar, hyaluronic acid, and soy protein *etc.* have been used for the fabrication of hydrogels due to their hydrophilicity, ionic character, and ability of chemical modification.⁸⁹

Among these polysaccharides, xanthan gum as a thickening agent, emulsifying, flocculating, and dispersing agent has been widely used for hydrogel formation. Bejenariu *et al.* synthesized xanthan hydrogels using adipic acid dihydrazide (ADH) as the crosslinking agent while 1-ethyl-3[3-dimethyl amino]propyl carbodiimide hydrochloride (EDCI) was used as a reagent in the reaction. Xanthan gum was dissolved in lithium nitrate (10^{-3} M) with magnetic stirring and the pH (3) was adjusted by using hydrochloric acid. After an hour, xanthan gum solution was heated up to the temperature of 90 °C, followed by the addition of ADH (molar ratio 2.5, 5 and 10) and EDCI (molar ratio – 1) and magnetically stirred for 24 h. The product was obtained in gel form, which was purified by dialysis in Milli-Q water and dried at a temperature of 40 °C and the synthesis yield (78%) was found with 25 g l⁻¹ xanthan gum concentration and ADH with 2.5 molar ratio. The rheological behavior of xanthan gum and xanthan hydrogels was similar as they showed the same helical structure and ordered confirmation. Elemental analysis of the hydrogels assured that the lowest ADH concentration (ADH : Xan-2.5) was able to confirm the possible reactions of all activated acid functions. Swelling studies carried out at pH 3, 7 and 13 showed that swelling increased with an increase in ADH : Xan ratio *i.e.* the highest swelling was noted at the ADH : Xan (10 molar ratio : 25 g l⁻¹) concentration due to the lowest crosslinking inside the gel. There was no considerable change with the change in pH, but higher swelling was noticeable at neutral pH. The hydrogels were loaded with methylene blue as a model molecule, by immersing the dried hydrogels in methylene blue solution for 24 h, with an encapsulation efficiency of 98%. Further investigation of the release kinetics of methylene blue from the hydrogels at pH 1.5 and 0.1 M NaCl revealed that the release of methylene blue is influenced by the method of synthesis of hydrogels.⁸⁸

Shalviri *et al.* (2010) evaluated cross-linked starch–xanthan gum hydrogels for drug delivery. The crosslinked hydrogels were prepared by preparing gels of starch (4%, w/v) containing varying amounts of xanthan gum (5–20% w/w of starch) and cross-linking with varying amounts of sodium tripolyphosphate (2–20% w/w of starch). The study reported that treatment of starch–xanthan gum hydrogels with sodium tripolyphosphate results in the cross-linking of starch, and phosphorylation with the formation of dephosphorylated polysaccharides. Furthermore, the cross-linking of starch and xanthan gum was proposed to form covalently linked hetero-polymer networks. It was observed that increasing the hydrophilic xanthan gum contents in the hydrogels increases their swelling. However,

increasing the cross-linking density of hydrogels by increasing the concentration of sodium tripolyphosphate also led to increased swelling. This increased swelling was ascribed to increased anionic phosphate groups on the hydrogels, which by electrostatic repulsion caused the expansion of the polymer network. The hydrogels were tested for drug release behavior by testing the permeability of a series of compounds with different molecular weight and charges, such as caffeine, FITC-dextran, ibuprofen, methylene blue, pyrogallol, sodium salicylate, vitamin B12, and verapamil. The results unveiled that the permeability of the compounds through the hydrogels was inversely proportional to their mol weight, while the permeability of negatively-charged molecules was less than the permeability of neutral or positively-charged molecules. The effect of molecular charge on permeability was explained to be due to repulsion of the negatively-charged drug molecule by the anionic polymer network which retarded the drug release. The study concluded that the cross-linked starch–xanthan hydrogels could be used for designing controlled release formulations of ionizable drugs.⁹⁴

In another study, Sethi *et al.* (2020) fabricated acrylic acid-grafted xanthan gum–starch cross-linked hydrogels using *N,N'*-methylenebisacrylamide (MBA) as the cross-linker and employing a microwave-assisted grafting reaction. For hydrogel fabrication, an initially homogenous mixture of xanthan gum and starch in water was prepared then ammonium per sulfate (APS) and MBA were added followed by the addition of a specific amount of acrylic acid with vigorous stirring. The synthesis of the hydrogels was optimized and the hydrogels were loaded with aspirin and paracetamol using a swelling diffusion method. The study reported that the swelling capacity of the hydrogels was more in pH 7.4 than in distilled water and pH 1.2. It was observed that the cross-linked hydrogels were thermally more stable and mechanically stronger than the individual polymers. Furthermore, the hydrogels were found to sustain the release of both paracetamol and aspirin. The release of drugs was pH-dependent which was attributed to the pH-dependent swelling of hydrogels and the pH-dependent solubility of drugs. The hydrogels were found to be non-hemolytic and non-cytotoxic.⁹⁵Table 13.2 details a number of research studies on xanthan gum hydrogels or nanocomposite hydrogels which have been explored for drug delivery applications.

Table 13.2 Xanthan gum-based hydrogel for drug delivery applications.

Composites	Method of preparation	Properties	Advantages/applications	Ref.
Xanthan–chitosan hydrogels	Chitosan and xanthan gum solution were mixed together to prepare hydrogel capsules. Furthermore, theophylline drug was added to prepare aqueous polyelectrolyte complex hydrogel capsules	Hydrogel capsules of diameter 3 mm were obtained. Xanthan : chitosan matrices of mass ratio 2.2 were found to be stable under acidic conditions and had sufficient swelling capacity	– Stable in both dissolution medium (pH 1.5 and 7.4) – Enhanced bioavailability – Release up to 12 h in a sustained manner	96
Acyclovir-incorporated chitosan–xanthan hydrogels	Free radical polymerization technique was utilized to formulate polymeric hydrogels	Antiviral drug was encapsulated with a content of 90.64% and	– Highly thermal stable	91

		release up to 87.75% drug at pH 7.4. Swelling ability of hydrogels was also found more in pH 7.4 than pH 1.2	– Non-toxic and biocompatible – Gastroretentive drug delivery	
Chitosan/dialdehyde xanthan blended hypromellose hydrogel	Synthesis of biopolymeric-based hydrogel was carried out using dialdehyde xanthan gum and chitosan. Then multiple drugs were added in the formulation such as ampicillin trihydrate, minocycline hydrochloride, and rifampicin	Cross-linking agent improve the gelation time, swelling, and gelling ability of the prepared hydrogels. Moreover, highly porous, mechanically, and thermally stable hydrogels represent controlled release of incorporated antibiotic drugs	– Excellent antibacterial activity against Gram-positive and Gram-negative bacteria – Sustained drug release of all drugs – Biocompatible and biodegradation capability – Suitable for drug delivery and tissue regeneration	97
Gentamicin-added xanthan gum–succinic anhydride hydrogels	High gel performance hydrogels were synthesized by mixing two polymers and then antibacterial agent was added	Addition of xanthan gum improved the rheological property and swelling degree of the formulation. With this, the release of gentamicin drug also occurs in a controlled manner	– Ionic strength-sensitive drug delivery system up to nine days – Better antibacterial activity against Gram-positive bacteria	98
Biodegradable stimuli-sensitive xanthan gum-based hydrogels	Co-polymerization of xanthan gum with poly(acrylic acid) was carried out to synthesize hydrogels under microwave irradiation	Swelling ability of hydrogels in distilled water and different salt solutions were found to be compatible	– Anti-bacterial against <i>Bacillus subtilis</i> and <i>Salmonella enteritis</i>	99

			– Biodegradable, non-toxic, and controlled release devices	
Carboxymethyl xanthan gum–trimethyl chitosan hydrogel	Modified xanthan gum and chitosan were mixed in aqueous phase and then ciprofloxacin was added	Ciprofloxacin was uniformly encapsulated (93.8%) and released (96.1% within 150 min) without a functionality change or structural integrity change	– Antibacterial – Cell viable and non-toxic – Controlled release formulation	100
Xanthan gum–poly(acrylic acid) semi interpenetrating polymer network (IPN) hydrogels	Using 1-butandiol dimethacrylate, potassium persulfate and hexamethylenediisocyanate, a complex network of natural and synthetic polymers was prepared <i>via</i> condensation reaction	Porous and mechanically strong IPN hydrogels exhibit appropriate hydrophobicity and better stability due to the cross-linking agent	– Suitable for drug delivery systems – Swellable at different pH values – Non-cytotoxic and cell viable	101
Xanthan gum-based hydrogels containing nanocapsules	Diphenyldiselenide and xanthan gum containing hydrogels were formulated into a nanocapsule suspension by interfacial deposition of polymers	A bright yellow colored hydrogel nanocapsule of diameter 280 nm and PDI 0.28 was obtained	– Antitumor effect against melanoma cell lines – Easy spreadability and cutaneous biocompatibility – Better skin permeation activity	102

13.3.4 Polyelectrolyte Complex

Polyelectrolyte complexes are 3D networks prepared by electrostatic interaction between oppositely charged species (*i.e.*, polyelectrolyte–drug, polyelectrolyte–polyelectrolyte, polyelectrolyte–nucleic acid, or polyelectrolyte–nucleic acid). The advantages of these association complexes like biocompatibility, stability, and biodegradability make them a promising material for various drug delivery systems. The natural gum-based polyelectrolyte complex and their composite hydrogels, films, tablets, beads, microparticles, and microspheres are well known pharmaceutical formulations

present in the market.¹⁰³ Xanthan gum-based polyelectrolyte complexes are also being researched for drug administration. Recently, Ciric *et al.* prepared the polyelectrolyte complex of two natural polymers *i.e.*, chitosan (cationic) and xanthan gum (anionic). Chitosan and xanthan gum solution (1 : 1, 1 : 2, 1 : 3) were prepared separately in acetic acid solution and purified water respectively. After that, ibuprofen was dispersed in polymeric solution at pH 4.6 and 5.6 using a homogenizer to obtain the final polyelectrolyte complex. The effect of pH on the different ratios of the polymer mixture was studied which revealed that most extensive cross-linking and high apparent viscosity occurred at pH 4.6 using a 1 : 1 chitosan to xanthan gum dispersion. However, higher proportions of xanthan gum (1 : 2 and 1 : 3) showed high % yield and drug entrapment efficiency than in proportion of 1 : 1. Also, the addition of poorly soluble ibuprofen drug decreases the conductivity and free charge mobility of the polyelectrolyte complex due to non-covalent interactions between the drug and polymers. Needle-shaped or quadrangular complex particles of length 22.44 μm to 258.50 μm were observed with semi-crystalline nature. Furthermore, the polyelectrolyte complex showed sustained release of ibuprofen up to 67.6% in 12 h which confirm that the prepared formulation can be used in oral administration for chronic therapy in inflammatory conditions.¹⁰⁴ Though the polymeric electrolyte complex can be used for drug administration, its formulation is also highly recommended due to enhanced pharmaceutical applications. Potas *et al.* synthesized the xanthan gum, tragacanth gum, and chitosan-based polyelectrolyte complex and its composite hydrogels for buccal drug delivery applications. It was noticed that the addition of xanthan gum in hydrogels significantly enhanced the mechanical strength which resulted in viscous and homogenous formation of hydrogels. Additionally, the incorporation of xanthan gum also affects the electrokinetic behavior of the formulation which leads to the zeta potential of 27.2 mV and conductivity of 0.27 mS cm^{-1} . The mucoadhesiveness of polymeric complex hydrogels was also improved due to xanthan gum. All these advantageous properties of polyelectrolyte complex-based hydrogels make them suitable candidates for bucoadhesive drug delivery systems.¹⁰⁵

A combination of modified cationic polymer (trimethyl chitosan) and pure anionic polymer (xanthan gum) is being researched for protein delivery in the gastrointestinal tract. BSA was selected as the protein model and encapsulated in the trimethyl chitosan and xanthan polyelectrolyte complex. A high amount of BSA concentration in the polyelectrolyte complex increases the encapsulation efficiency of the protein due to the strong electrostatic interaction force between the polymer and drug. The swelling ability of the polymeric complex without BSA was evaluated against pH 1.2 and 7.4 which inform that at alkaline pH 7.4, the swelling rate is high as compared to acidic pH 1.2 due to electrostatic repulsion between protons and the hydroxyl group. Similarly, *in vitro* release of BSA in pH 7.2 was found more optimum than in pH 1.2, followed by a non-Fickian mechanism. In addition, no cytotoxicity, better cell viability, and good biocompatibility of the polyelectrolyte complex was examined.¹⁰⁶ Figure 13.3 portrays the numerous applications of xanthan gum-based formulations.

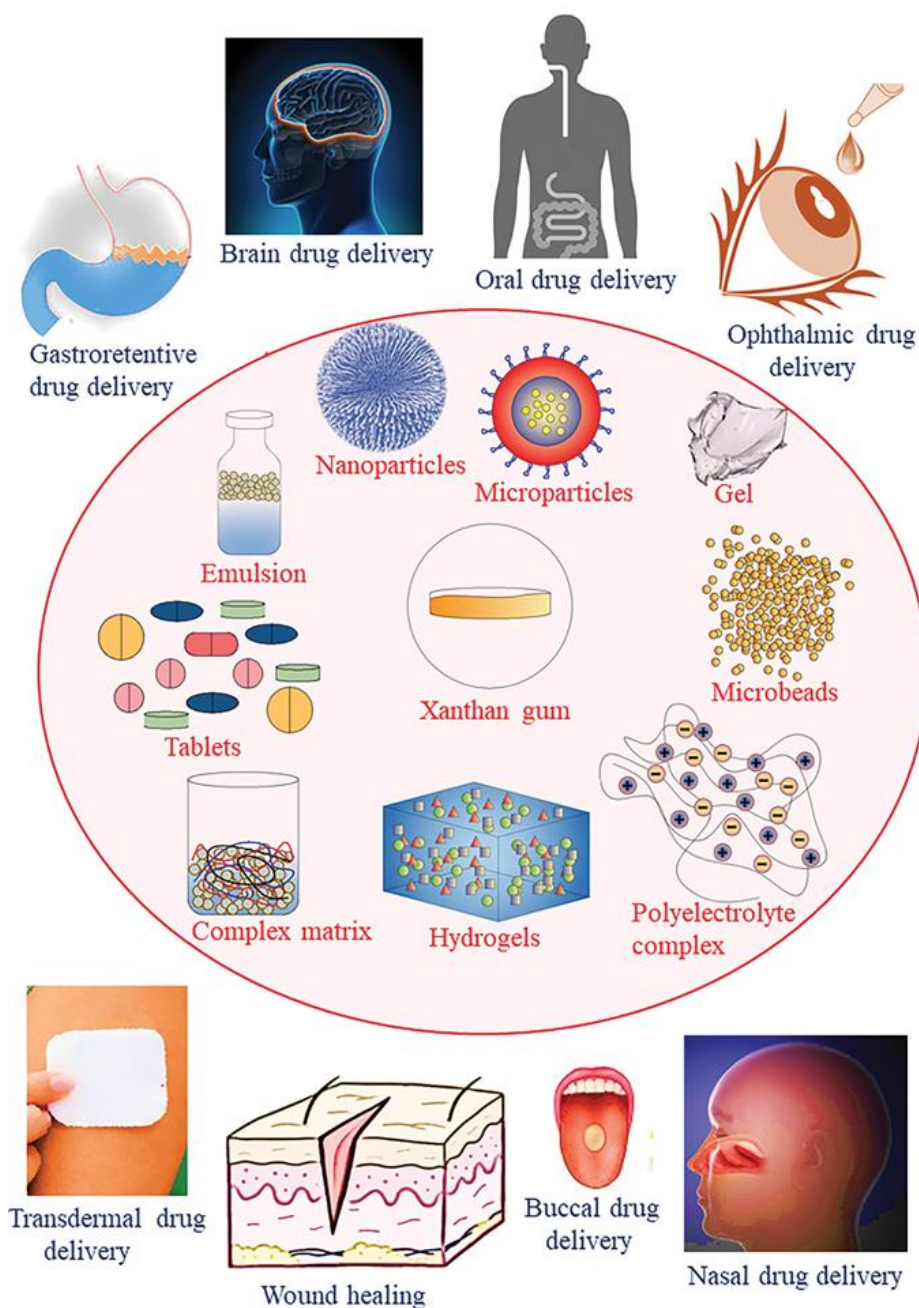


Figure 13.3 Various formulations of xanthan gum for drug delivery applications.

13.3.5 Tablets

The use of a biopolymer matrix system is always appropriate as a carrier for the development of oral formulations. The swellable nature of natural gums render them suitable for formulation of sustained drug delivery. Jian and co-workers prepared xanthan gum tablets using a wet granulation technique by employing xanthan (main ingredient), lactose (filler), extracted galactomannan (excipient), magnesium stearate (lubricant), and theophylline (drug). The matrix tablet with a diameter of 12 mm and height of 4.5 mm was obtained with an effective drug amount (99.8–100.4%). The drug release

property of xanthan gum tablets was evaluated in acidic pH (1.2) and alkaline pH (6.8). It was found that the theophylline drug showed a more sustained release behavior from combined gum tablets than a single gum tablet. The drug release mechanism was found to be non-Fickian diffusion or anomalous transport. The whole study provided strong evidence that a xanthan gum-based matrix can be used as a novel material for controlled anti-hypertensive drug delivery at the colon target site.¹⁰⁷ Aside from pure xanthan gum, modified xanthan gum is also being explored in tablet manufacturing. Kumar and their team firstly grafted the xanthan gum employing poly(acrylamide) by using microwave-assisted synthesis. The grafted xanthan gum was formulated into matrix tablet for further usage. Diclofenac sodium drug-loaded grafted xanthan gum-based matrix tablets were found to be reproducible and effective. The marketed diclofenac tablet showed 100% drug release in 10 h, while the formulated tablet released only 49% of the drug in 16 h which clearly indicated that the grafted xanthan gum controls the release property of the matrix tablet. In a similar study by Mundargi *et al.* (2007), acrylamide grafted xanthan gum was composed into a copolymer-based matrix tablet in which antihypertensive drugs atenolol and carvedilol were incorporated using microcrystalline cellulose, poly(vinylpyrrolidone), and grafted xanthan gum. The formulated copolymer tablet exhibited 85% release in 24 h while pure xanthan gum-based tablet released 99% drug in 12 h only. The overall results of the prepared formulation depicted that a matrix tablet of two or more natural polymers are suitable hydrophilic carriers for designing oral controlled drug delivery systems.³³

13.3.6 Gels

The residence time of viscous *in situ* gel or dispersion is sufficient for ophthalmic drug delivery systems. Natural polymers are a favorable material for preparing *in situ* gels as they have an effective swelling capability, stability, and easy shape-holding properties. Xanthan gum is one of the promising polymers because it has better stabilizing, thickening, rheological, and mucoadhesive properties. Interestingly, “Sensodyne®” toothpaste gel marketed by “GlaxoSmithKline Pharmaceutical Company” also includes xanthan gum in its composition. Ceulemans and their co-researchers initially explored the significance of xanthan gum for ophthalmic drug delivery and its significant effect with mucin. The effect of polymer concentration, mucin concentration, and boiling/sonication time on the xanthan–mucin dispersion was investigated by determining the change in rheological properties. The study illustrated that 1% of xanthan and 8% of mucin is sufficient for obtaining a stable and viscous ocular dosage form with a sonication time of 2 min at 80 watts.¹⁰⁸ An ophthalmic gel of xanthan gum using sodium hyaluronate has also been investigated by researchers for corneal wound healing applications. The gel formulation of xanthan gum (1%) and sodium hyaluronate (0.15%) was evaluated against corneal epithelium for the correction of myopia following photorefractive keratectomy. It was observed during the investigation that the produced polymeric gel was well suited and highly effective for the treatment of corneal wounds in just three days with complete healing in nine days. Similarly, Faraldi and the team synthesized an eye gel of xanthan gum (1%) employing sodium hyaluronate (0.15%) and netilmicin (0.3%). The eye gel was further used for the treatment of post-traumatic corneal abrasions.¹⁰⁹ Not only is a single natural polysaccharide used to prepare the *in situ* gel for ophthalmic drug delivery but two or more than two polymers can also be combined to design a topical ophthalmic delivery system. On the basis of this, Hiremath and co-workers prepared a polymeric dispersion of hydroxypropyl guar, xanthan gum, hydroxyethyl cellulose, cyclodextrin, sodium alginate, and Carbopol 934. Linezolid drug was loaded during formulating an *in situ* gel for better therapeutic activity. Analysis of the formulated gel revealed that a combination of multiple polymers in the drug-loaded ophthalmic gel releases the drug in a sustained manner and increased the residence time with better therapeutic efficacy and bioavailability.¹¹⁰

Recently, a similar formulation was prepared by Zhang and his team in their study except for the usage of many polymers. Linezolid added ophthalmic solution of xanthan gum was produced using an autoclaving technique. The obtained clear and transparent solution represented good viscosity and excellent content uniformity. In addition to this, the synthesized ophthalmic dosage form showed non-cytotoxicity, extensive corneal permeation, good ocular tolerance, and great antibacterial activity.¹¹¹

13.3.7 Complex Matrix

Despite simple and easily processed xanthan formulations, a complex matrix has also been prepared with enhanced physico-chemical properties. Petitjean and Isasi (2021), synthesized the hydrogel matrices of natural polysaccharides (chitosan, xanthan gum, and locust bean gum) cross-linked with or without β -cyclodextrin using citric acid. Disodium hydrogen *ortho*-phosphate was used as the catalyst in the cross-linking reaction. Ratios of polysaccharides, citric acid, and β -cyclodextrin were taken according to the ternary plot. All the reagent mixture was crushed and baked at 170 °C for 20 min in an oven. The obtained product was washed with deionized water, filtered, dried, and powdered into matrices. The maximum yield of xanthan gum matrices was found to be 60% while using chitosan and locust bean gum the yield was 70% and 30% respectively. Swelling of xanthan gum matrices was higher than other matrices, which can be due to the various acidic functional groups present in the structure. Xanthan gum cross-linked with β -cyclodextrin showed the maximum absorption of phenolphthalein due to a high number of β -cyclodextrin cavities. Sorption of 1-naphthol was high in locust bean gum matrices with low β -cyclodextrin cavities while chitosan matrices with a high ratio of β -cyclodextrin showed progressive sorption of 1-naphthol. Xanthan gum exhibited the same sorption with all compositions. All polysaccharides showed differences in the cross-linked or without cross-linked matrices.¹¹²

Anghel *et al.* (2021), also presented the application of a xanthan matrix for drug delivery application by carrying out the esterification of xanthan gum with acrylic acid as discussed in Section 13.2.4.⁴² Maity and Sa investigated the calcium cross-linked carboxymethyl xanthan gum mini matrices for the effect of calcium ion concentration on the swelling property and mechanism of drug release. The modified carboxymethylated xanthan gum (CMXG) was mixed with calcium chloride solution to form a cohesive mass which was passed through a sieve to obtain granules. Magnesium stearate was mixed with granules and these granules were further compressed in the form of mini-matrices by using a minipress tablet machine. The final obtained calcium cross-linked CMXG mini matrices showed less swelling which could be due to the viscous gel layer formed around the matrix by preventing the water penetration. From a low concentration to high concentration of calcium ions, the release rate decreased but at a higher concentration of calcium ion, the release rate increased, which occurred due to matrix erosion. The determined results indicated that cross-linked complex matrices can be used as drug delivery systems with different types of drug release mechanisms.¹¹³

Scrutinizing all the advantageous properties and beneficial formulation of xanthan gum, we can easily conclude that xanthan gum or its derivatives can be used extensively for pharmaceutical, food, and cosmetic applications.

13.4 Conclusion

Xanthan gum is one of the widely used natural polysaccharides in drug delivery due to its numerous advantages like non-toxicity, biocompatibility, biodegradability, and stability. Several research articles of xanthan gum are present on different search engines. The gum-based pharmaceutical formulations such as tablets, beads, gels, micro or nanoparticles, and polyelectrolyte complexes have

been investigated thoroughly. Furthermore, physico-chemical modifications of xanthan gum to impart specific properties have enhanced its applications. The advantageous characteristics of obtained xanthan formulation, make it a reliable carrier in drug delivery dosage forms. Xanthan gum has found applications as a drug carrier for oral, mucosal, nasal, transdermal, gastroretentive, and ophthalmic drug delivery systems. Furthermore, there exists a lot of scope in exploring xanthan gum for protein or gene delivery and in 3D-printing applications.

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Chapter 14

Cellulose-based Biomaterials in Drug Delivery Applications

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14.1 Sources of Cellulose Biopolymer

Cellulose is the most abundant biopolymer on earth.¹ It is the major component of the plant cell wall and the most abundant component in wood. The primary and tertiary (lumen) cell walls of plants are formed by a thin layer (100–200 nm thickness) of microfibril disordered cellulose, pectin, and hemicellulose, while the secondary cell wall is composed of three layers, the first (S1) and the third (S3) with a thickness in nanometric order and the second (S2) in micrometers, this layer is formed by cellulose microfibrils arranged parallel and helical, oriented by the microfibril angle (see [Figure 14.1](#)).² The orientation of cellulose fibrils in the cell wall is important due to the control of resistance and deformation, and the mechanical properties. It is described in the literature that the microfibril angle and the mechanical properties are different for each botanical species and in general there is an inversely proportional relation of the microfibril angle with mechanical resistance.³

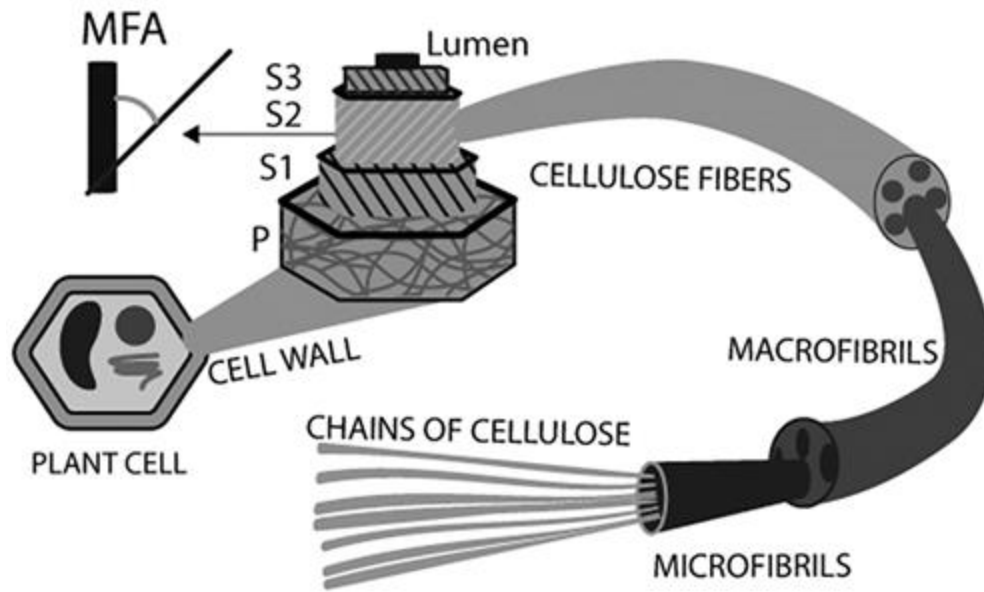


Figure 14.1 Cell wall illustration, P = primary wall, S1–S3 primary, secondary, and tertiary cell wall, MFA: microfibril angle and grading of cellulose fibers to molecular chain.

Some bacteria can produce cellulose from biosynthesis. In 1886 Brown pioneered the identification and characterization of bacterial cellulose (BC) production by the bacteria of the genus *Komagataeibacter xylinus* (formerly called *Acetobacter xylinum*) in the presence of oxygen and sugar.⁴ In contrast to cellulose from plant origin, BC has high purity because it is free of components such as lignin, hemicellulose, and pectin, among others, besides having 84–90% crystallinity, while cellulose of plant origin has values of 40–60%.⁵ In the initial (never dry) state BC consists of a three-dimensional network containing 99% water and cellulose nanofibrils 80–150 nm.⁶

Tunicates (subphylum of *Urochordata*) are the only animal group whose tissues are formed essentially by microfibrils of cellulose and proteins.⁷ The cellulose microfibrils extracted from the tunicates have a crystallinity of approximately 95%.⁸ In addition to plant varieties, bacteria and tunicates cellulose can be isolated from algae (yellow, green, red, and brown species). [Figure 14.2](#) shows some sources of cellulose such as wood, cotton, bamboo, bacteria, algae, and tunicates.

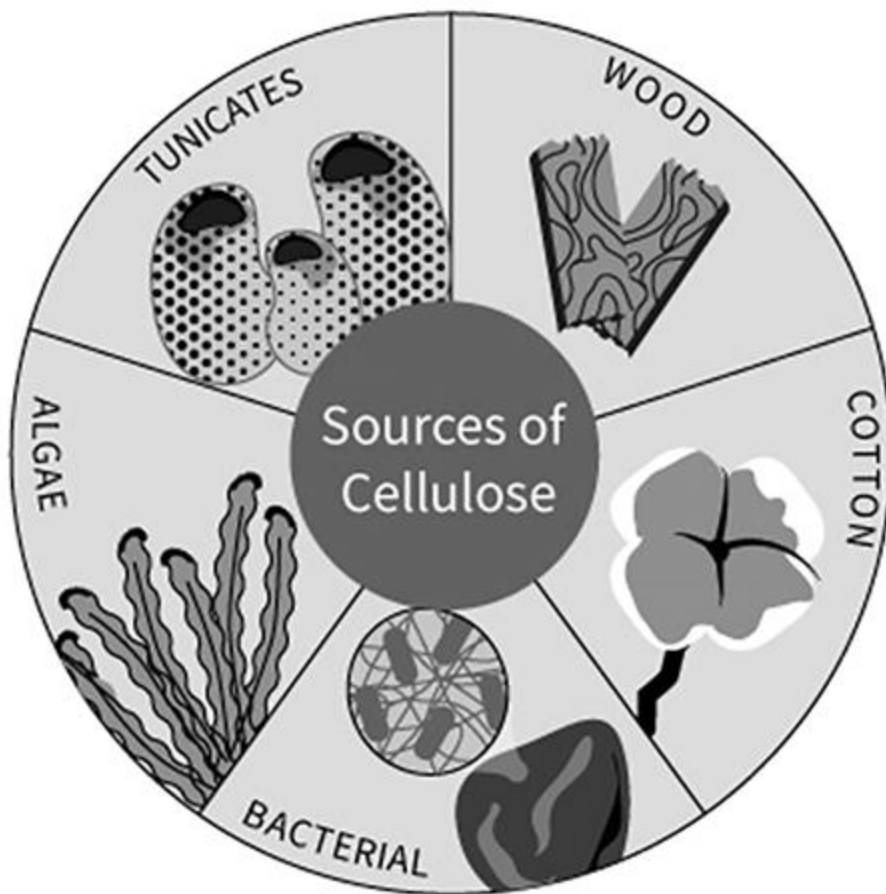


Figure 14.2 Main cellulose sources: wood, cotton, bacteria, algae, and tunicates.

14.1.1 Cellulose Structure

Cellulose is a polysaccharide and independent of source the structure consists of $4C_1$ chair conformation linked β - α -glucopyranose units connected by 1,4-glycosidic bonds, resulting in an alternating turn of 180° under the axis of the cellulose chain. The repetitive units of α -glucopyranose are called anhydroglucose (AGU) in cellulose, and present three groups of reactive hydroxyls, in the primary carbon (C6) and in the secondary carbons (C2 and C3). The hydroxyls are projected in the plane of the ring and two units of AGU are considered the repetitive unit of cellulose, the cellobiose⁹ (see [Figure 14.3](#)).

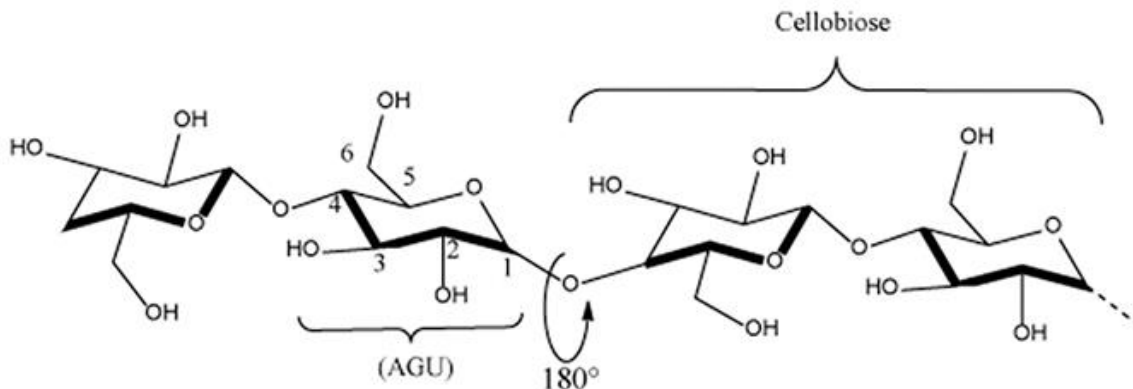


Figure 14.3 Schematic representation of the cellulose structure.

Solubility, chain stiffness, reactivity of hydroxyl groups, crystallinity, and some other properties of cellulose are influenced by hydrogen bonds.¹⁰ These bonds are formed by the three hydroxyl groups of the AGU and the oxygen of the α -glucopyranose ring. The cellulose has two sites of intramolecular hydrogen bonds, which are, the one between the hydrogen of the HO-R belonging to the C3 with the adjacent oxygen of the α -glucopyranose ring, and between the oxygen of the C6 hydroxyl with the hydrogen of the C2 hydroxyl. These intramolecular bonds are responsible for the rigidity of the chain, and intermolecular bonds occur between the polysaccharide chains, responsible for the high interaction, arrangement, and crystallinity.⁹

14.1.2 Cellulose Allomorphs

Due to the intermolecular bonds, there are different possibilities of connections between cellulose chains that could originate from allomorphs, and the main cellulose allomorphs were classified as I, II, III and IV. Cellulose allomorph type I (CI) exists in two forms $I\alpha$ and $I\beta$ with a triclinic unit cell and monoclinic unit cell, respectively.¹¹ Both forms coexist and the ratio varies from each source; in plants and tunicates the highest fraction is $CI\beta$, while in bacteria, $CI\alpha$. Through irreversible reactions, such as regeneration or mercerization, cellulose I is transformed into cellulose II (CII), so CII is thermodynamically more stable than CI. Cellulose type II (CII) has no record of natural origin, being formed through regeneration or

mercerization reactions, and it has a monoclinic unit cell with two antiparallel chains.^{2,12} The mercerization process involves treatment with high concentrations of aqueous sodium hydroxide, followed by washing and recrystallization,¹³ whereas regeneration occurs by dissolving the cellulose in some solvents followed by recrystallization. Different solvents have already been reported for CII regeneration being mainly phosphoric acid,¹⁴ ionic liquids¹⁵ based mainly on alkylammonium cations, alkyl phosphonium, 1-alkyl pyridinium, and 1,3-dialkyl imidazolium combined with different anions such as Cl^- , NO_3^- , $[\text{BF}_4]^-$, and CF_3COO^- among others. Allomorphs cellulose III is obtained by treatment of CI or CII with ammonia and amines, generating CIII_I (parallel chains) or CIII_II (antiparallel chains) respectively. According to Sarko *et al.*,¹⁶ CIII_I and CIII_II have the same unit cell. With treatment with glycerol at high temperatures (260 °C) CIII is transformed into cellulose IV, also in two forms, IV_I and IV_II , depending on the form of the cellulose origin, CIII_I or CIII_II . After thermal treatment of cellulose type III, followed by recrystallization, it is possible to convert both CIII and CIV into the initial cellulose (CI or CII), an example of cellulose allomorph reversibility.¹⁷ Figure 14.4 shows a scheme of the reactions and allomorphs of cellulose.

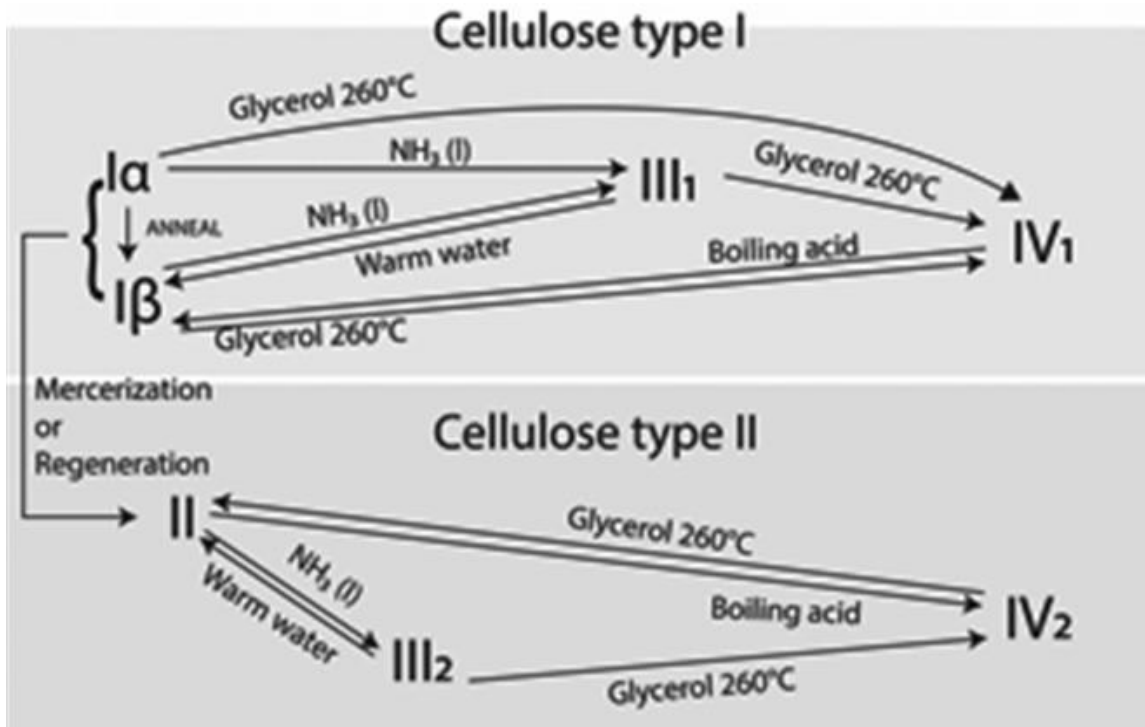


Figure 14.4 Diagram of cellulose allomorph conversions.

14.1.3 Characterization of Allomorphs

Structural investigations are usually performed by X-ray diffraction,¹⁸ allowing the characterization of cellulose allomorphs from the diffractogram based on peak position, intensity, diffraction peak width, planar distance, crystallinity, crystal sizes, and network distortion. The diffractogram is usually obtained by scanning 2θ from 6° to 50° , using $\text{CuK}\alpha$ -radiation ($\lambda = 0.15418 \text{ nm}$), using the reflection mode.

14.2 Cellulose Classification

14.2.1 Microcrystalline Cellulose (MCC)

MCC is usually obtained from wood and cotton due to their abundance, but at high cost based on market availability. Recent research seeks to produce MCC from other sustainable sources, for example, coffee husk¹⁹ and cotton waste.²⁰ Different methods such as acid hydrolysis HCl , H_2SO_4 , extrusion,

and enzymatic processes are reported for isolation of MCC. Independent of sources MCC belong to polymorph cellulose type I and 60–70% crystallinity index.²¹

14.2.2 Cellulose Nanofibrils (CNF)

CNF exist in nature in different sizes (length 20–50 nm and width 500–2000 nm), associated with the presence of cellulose microfibrils (CMF), which are composed of CNF and both are used as synonyms in the literature. Due to the organization of the cellulose chains CNF maintain the crystalline and amorphous domains and are more commonly used after some surface treatment.² Usually obtained by mechanical treatment, fibrils are highly entangled and this network brings unique characteristics to CNF such as high strength and low concentration gel formation.²²

14.2.3 Cellulose Nanocrystals (CNC)

The cellulose is formed by regions of high ordering, located in crystalline regions and areas with low ordering, giving rise to amorphous regions, which can be hydrolyzed with acid and resulting in crystalline fragments, called CNC or nanocrystals of cellulose (NCC). The hydrolysis of cellulose fibers catalyzed with sulfuric acid was first reported by Rånby in the 1950s obtaining a colloidal suspension of cellulose nanocrystals.²³ Different acids can be used for cellulose hydrolysis reactions, and the classics are sulfuric acid, phosphoric acid, and hydrochloric acid.^{24–26} Usually the NCC isolated through acid hydrolysis present rod-like morphology, also referred to as whiskers, with lengths ranging from 200 to 400 nm and widths less than 10 nm,²⁴ but the size strongly depends on its source.

14.3 Cellulose Derivatives

As mentioned in Section 14.1.1, the groups responsible for intra- and inter-molecular interactions are the hydroxyls of the AGU and when available on the polymer surface can react under different chemical reactions and conditions. Reactions can occur in homogeneous or heterogeneous conditions. Homogeneous reactions occur in a single phase, where the

cellulose is completely soluble in the solvent. The main advantage of this condition is that the hydroxyl groups are completely accessible. Whereas reactions under heterogeneous conditions, in which cellulose is in the solid state (suspension) the full access of hydroxyls is not allowed, therefore in this condition the main advantage is the reactions in a limited number of free hydroxyls on the surface, thus preserving the inner crystalline structure of the cellulose.⁹

14.3.1 Cellulose Ethers

Because they are non-toxic and soluble in water, ethers have several applications, in the pharmaceutical, food, paint, paper, cosmetics, agriculture, textile, and other industries. Several methods can be employed to synthesize cellulose ethers (see Figure 14.5), by common reactions of organic chemistry such as Williamson syntheses (alkyl or aryl halides) in the presence of strong base, ring opening (epoxy) and Michael's addition.

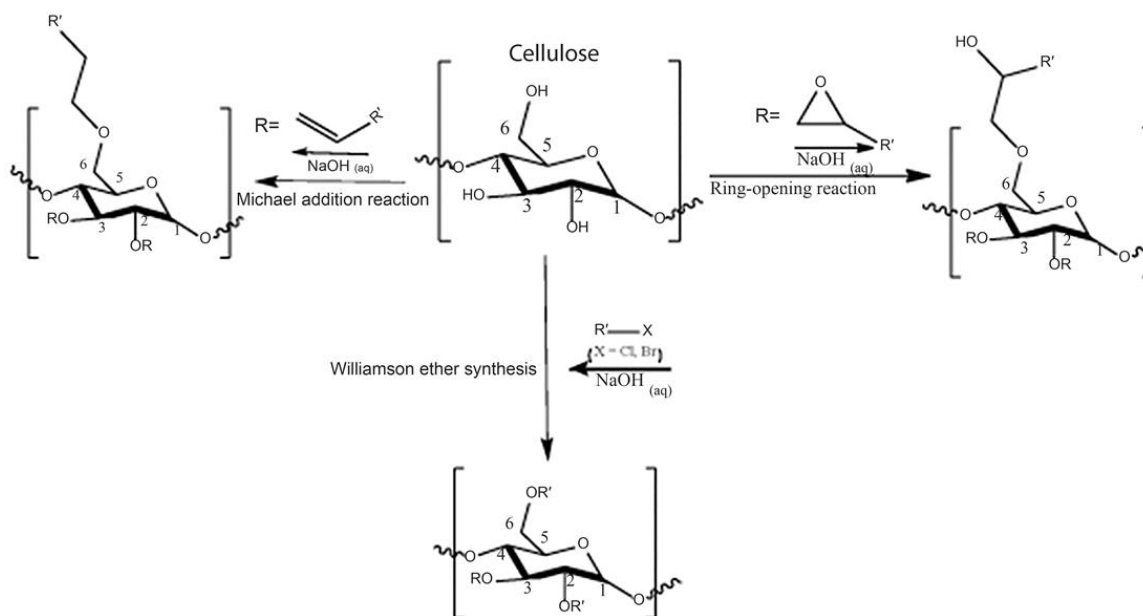


Figure 14.5 Schematic representation of cellulose derivative reactions.

These cellulose derivatives are classified into ionic and non-ionic (see Figure 14.6).

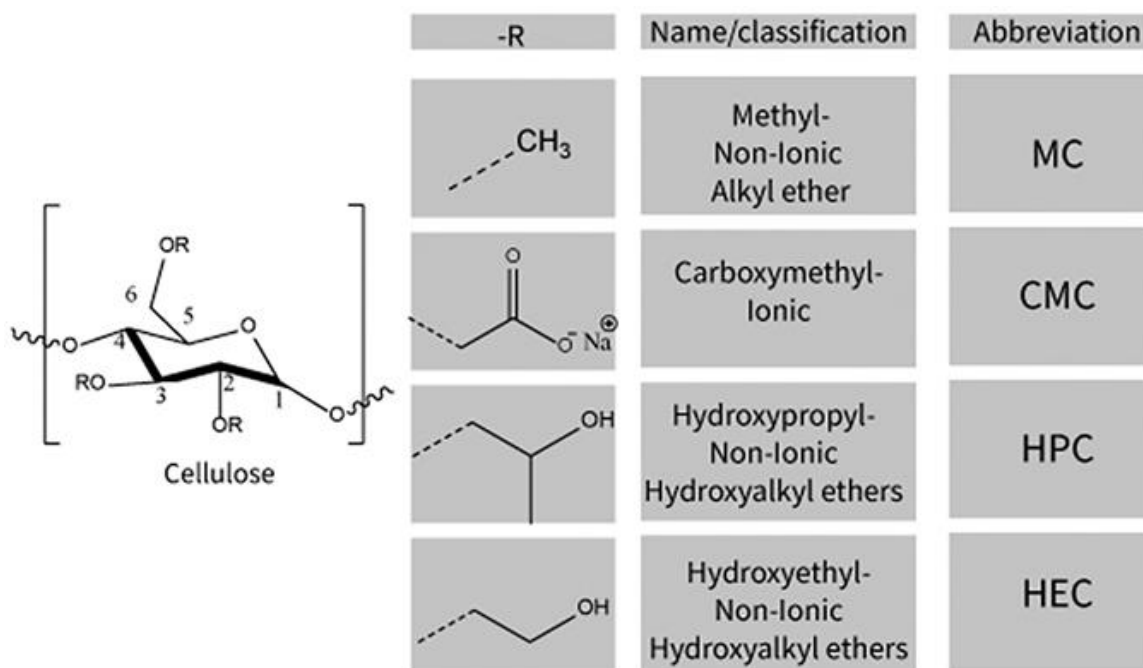


Figure 14.6 Schematic representation of some cellulose derivatives.

14.3.1.1 Methylcellulose (MC)

MC, classified as a nonionic cellulose alkyl ether, is thermoplastic and can be dissolved in different organic solvents. In addition to the solubility another important characteristic of this derivative is viscosity, and commercially MC is produced with different molar masses that can be expressed in terms of apparent viscosity values. Polymer synthesis is by heterogeneous condition followed by methylation or by homogeneous conditions with aqueous NaOH/urea solution and subsequent methylation.²⁷ The distribution of methyl groups in the AGU influence the properties of MC, and the methylation reaction is regioselective with C2 > C3 > C6 as the preferred substitution order.²⁸ Cellulose alkyl ethers, as MC, solubilize at room temperature, but form gels under heating, and these thermoreversible gels have been applied in pharmaceutical areas, as drug delivery agents.²⁹

14.3.1.2 Carboxymethylcellulose (CMC)

CMC is synthesized by reacting cellulose using monochloride acetic acid or the sodium salt, in the presence of aqueous NaOH solution. The degree of substitution along the chain depends on the conditions employed during the reaction such as time, temperature, reagents, and if the reaction was performed under homogeneous or heterogeneous conditions. AGU hydroxyls C2, C3, and C6, are susceptible to carboxymethyl introduction reaction, so CMC may contain different degrees of substitution (replaced at all points, or at some points), and the favored reaction is $C6 > C3 > C2$ under homogeneous and $C2 = C6 > C3$ under heterogeneous conditions.^{30,31}

14.3.1.3 *Hydroxyethyl Cellulose (HEC) and Hydroxypropyl Cellulose (HPC)*

HEC and HPC cellulose derivatives classified as hydroxyalkyl ethers have common properties with alkyl ethers, but HPC forms a thermoreversible gel while HEC does not. The hydroxyalkyl ethers are synthesized by reaction of the cellulose with epoxides that react through the ring opening in alkaline conditions (NaOH solutions). Under heterogeneous conditions with oxirane in a “slurry process” the reactivity of the hydroxyls $C2 = C6 > C3$, and the reactivity of C2 and C6 varies with the concentration of NaOH. However, under homogeneous conditions with the use of ionic liquids the reported reactivity is $C2 > C6, C3$.^{27,32}

14.3.1.4 *Hydroxypropylmethyl Cellulose (HPMC)*

HPMC is another cellulose ether classified as non-ionic, and to synthesize it cellulose is treated in aqueous solution NaOH with methyl chloride and propylene oxide, and the hydroxyl groups are replaced randomly by O-2-hydroxypropyl and O-methyl. HPMC has biocompatible characteristics, a thermoreversible gel, water retention, an active surface, is adhesive, and an emulsifier among other things, and is widely used as a hydrophilic matrix for drug delivery systems, and cosmetic and food formulations.^{33,34} The HPMC characteristics are directly correlated with the proportion of the methyl and hydroxypropyl groups. Due to its applications in the pharmaceutical industry and randomness of group substitutions, the United

States Pharmacopoeia (USP) designates some types of HMPC, the first two being the methyl portion and the last two hydroxypropyl, with examples being HMPC 2208 and HMPC 2906.³⁵

14.4 Cellulose-based Gels in Drug Delivery

Cellulose is a very versatile biopolymer with numerous possibilities in drug delivery applications. Nowadays there are many studies aiming at its uses, especially in the form of gels for biomedical and drug delivery applications, since these materials are able to achieve some requirements for this purpose, such as stimuli-responsive systems.

14.4.1 Nanocellulose Hydrogels

Nanocellulose is one of the most widely used materials for drug delivery applications and comprehends three sub-classes of materials: cellulose nanocrystals (CNC), cellulose nanofibers (CNF), bacterial cellulose (BC)³⁶ and derivatives of cellulose.⁹ Hydrogels can be formed when the nanocellulose is dispersed in water and depending of the drying process can form xerogels, using ambient temperature drying, or aerogels, using techniques such as supercritical drying.³⁷

Hydrogels are also widely used biomaterials, since they are gel-like structures with functional groups, capable of holding large amounts of water.³⁸ These materials are formed from a mechanism known as gelation, since segments of the polymer chain can interact with each other up to the “gelling point”, where the relaxation time of the system becomes infinite and there is no further flow of the system.³⁹

Nanocellulose forms colloidal suspensions when dispersed in water, and the gels made of these materials can be formed by two mechanisms: (a) physical crosslink or (b) chemical crosslink. In the first case, the network is formed through electrostatic interactions between chain segments, including van der Waals forces, and ionic or hydrophobic interactions, all reversible interactions when these gels are broken down. Nanofibers or nanocrystals can have charged groups on their surface, which generates hydrogels through electrostatic interactions. On the other hand, networks can be formed through covalent bonds between the chains, which generate a strong

and permanent interaction. There are several ways to make these bonds, such as chemical crosslinkers like epichlorohydrin (ECH), metal ions, and numerous others.⁴⁰ Physical or chemical crosslinking points are formed between two polymer chains, creating the gel porosity, one of the most important properties of hydrogels, that is related to the crosslinking density (CD), reflecting on the pore size (ϵ), which is very important for applications in drug delivery⁴¹ (see Figure 14.7).

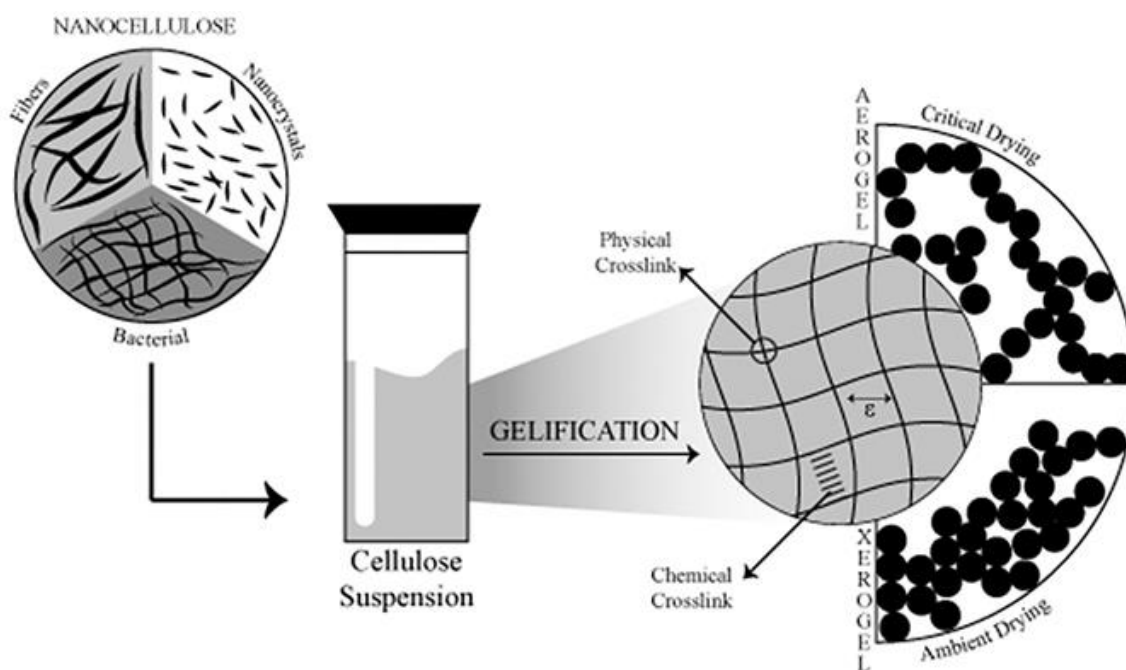


Figure 14.7 Nanocellulose, represented by bacterial cellulose, nanofibers, and nanocrystals in aqueous suspensions. Through a gelification process it can form physical or chemical crosslinks, forming networks that support the gel and govern the size of the network pores (ϵ) which are related to the physical properties of the gel. Aerogels are formed by drying under critical conditions and xerogels are formed when the solvent is evaporated at ambient conditions.

14.4.2 Hydrogels by Modified Cellulose

The surface of cellulosic materials can be modified by oxidation for example, which increases their affinity for water and increases their biodegradability. They have been greatly explored for drug delivery applications by increasing their compatibility with water and facilitating future chemical modifications of the surface that can make the hydrogels

protect the drugs until they reach their target, getting released in a controlled way under the effects of a stimulus.⁴² It is also possible to bind drugs on the backbone of polymeric chains through physical interactions or chemical bonds and to change mechanical properties with other polymers (see Figure 14.8).

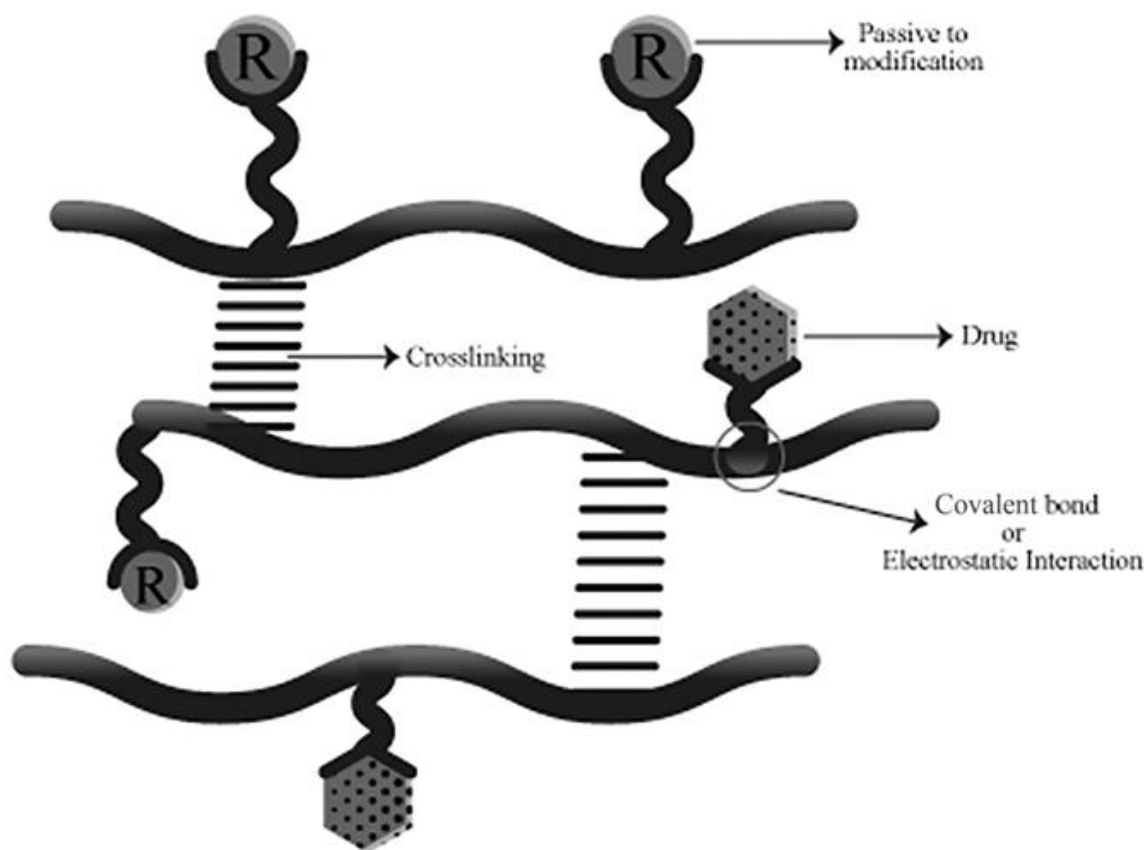


Figure 14.8 Polymeric cellulose chains can be functionalized through several chemical groups, changing their properties, or making them interactive with other chemical groups present in drugs. Drug molecules can also interact with the chain through chemical bonds or electrostatic interactions.

Barbu *et al.*⁴³ for example, oxidized cellulose nanofibers through a reaction with 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO). Subsequently this material was functionalized to allow folic acid to bind to the chains of the cellulose nanofibers. This modification enables the positive charges of the amino group of doxorubicin to interact with the negative charges present in the carboxylated cellulose matrix, through ionic interactions.

Liang *et al.*⁴⁴ have shown that aerogels can be formed from cellulose nanofibers responsive to temperature and pH and they may be applied in drug delivery.

Hydrogels can also be formed with copolymers. Song *et al.*⁴⁵ improved the mechanical properties of CMC by introducing a secondary chain of copolymers isopropyl acrylamide (NIPAM) and acrylamide (AM). The hydrogel formed responds to stimuli of pH and temperature, important factors for the process of drug delivery. The results showed that it is possible to release tetracycline at intestinal pH and physiological temperature accurately treating an intestinal bacterial infection. Furthermore, by changing the proportions between NIPAM and AM it is possible to alter the low critical solution temperature (LCST) of the gel, thus modulating the drug delivery system.

14.4.3 Cellulose Derivative Hydrogels

Capanema *et al.*⁴⁶ used the amino groups of doxorubicin to make a covalent bond with the carboxylic groups of CMC, generating a polymer–drug bioconjugate, where doxorubicin became covalently bound in the polymer chain, and to form the hydrogel, citric acid was used to crosslink the chains.

Another way to increase the mechanical strength of CMC-based hydrogels was explored by Jeong *et al.*⁴⁷ The authors used a dual-component system of β -cyclodextrin/CMC or beta-cyclodextrin into crosslinked CMC chains, which increased not only the mechanical strength, but also the gel swelling ability. These hydrogels also exhibit a better tetracycline-loading capacity than conventional ones.

It is also possible for drug release due to an electric field stimulus. Sangsuriyonk *et al.*⁴⁸ used CMC hydrogels as citric acid as a crosslinker, to result in a controlled transdermal release of 5-fluorouracil, an anticancer drug, by applying an electric field.

14.4.4 Cellulose Aerogels

Aerogels can also be formed from regenerated cellulose, after dissolution in ionic liquids, mercerization, or phosphoric acid. Those are solvents which can break the intramolecular hydrogen bonds present in the cellulose

chains, and when solubilized, the cellulose can be precipitated using an antisolvent such as water or ethanol. Lin and Jana⁴⁹ for example, used an ionic liquid mixture with dimethylformamide (DMF) to solubilize MCC that was precipitated in ethanol as an antisolvent. As a result, monoliths were obtained by solvent exchange and supercritical drying and aerogel microparticles from oil-in-oil emulsion, both candidates for drug delivery. Acetaminophen was used as the model drug, but the microparticles prevented the release of the drug and water ingress into the monoliths caused fracture of the internal structures.

Santra and Sen⁵⁰ solubilized MCC with ionic liquids and the solubilized cellulose was modified with tannic acid or L-methionine, and finally the selenourea was incorporated into the medium. The material was gelled in water and subsequently the solvent was removed resulting in spherical gels of cellulose. The release of selenourea was evaluated in different media and also in a simulated gastric environment. In addition, the tyrosinase inhibition was investigated with the release of selenourea. The tannic acid modified gel showed the highest release. However, the inhibitory activity for tyrosinase was comparable for all gels.

Xu *et al.*⁵¹ also used the regeneration technique to create hybrid starch (amylose/amylopectin) hydrogels with MCC. The polysaccharides, with different ratios, were dissolved in ionic liquid and regenerated in water as an antisolvent, and then the samples were freeze-dried. The obtained gels showed good buoyancy in a simulated gastric environment (pH 1.2) making them strong candidates for floating drug delivery systems. The authors propose that the drug delivery of ranitidine occurs by controlled diffusion and osmotic pressure mechanisms.

14.4.5 Cellulose Nanocrystal Gels

CNCs are commonly used as mechanical reinforcement agents in polymeric matrices.^{52,53,71,72} The influence of CNC and nanofibers on poly(vinyl alcohol) xerogel formulations were investigated by Pramanik *et al.*,⁵⁴ and the authors evaluated the concentrations of 7%, 13% and 18% of CNCs in the formulations, concluding that with the increase in the concentration of CNCs the mechanical strength increases, but when the concentration of cellulose nanofibers increases, the xerogels start to become fragile due to

the formation of a fibrous network. Therefore, the mechanical properties improved with the addition of nanocellulose, but concentrations higher than 18% of CNC and 13% of nanofibers make the xerogels more fragile. CNCs are versatile materials, since they have a large number of hydroxyls on their surface susceptible to countless ways of graphitization and functionalization, which allows CNCs to interact specifically with particular drugs or interact with segments of the polymeric network, changing their physical properties (see [Figure 14.9](#)).

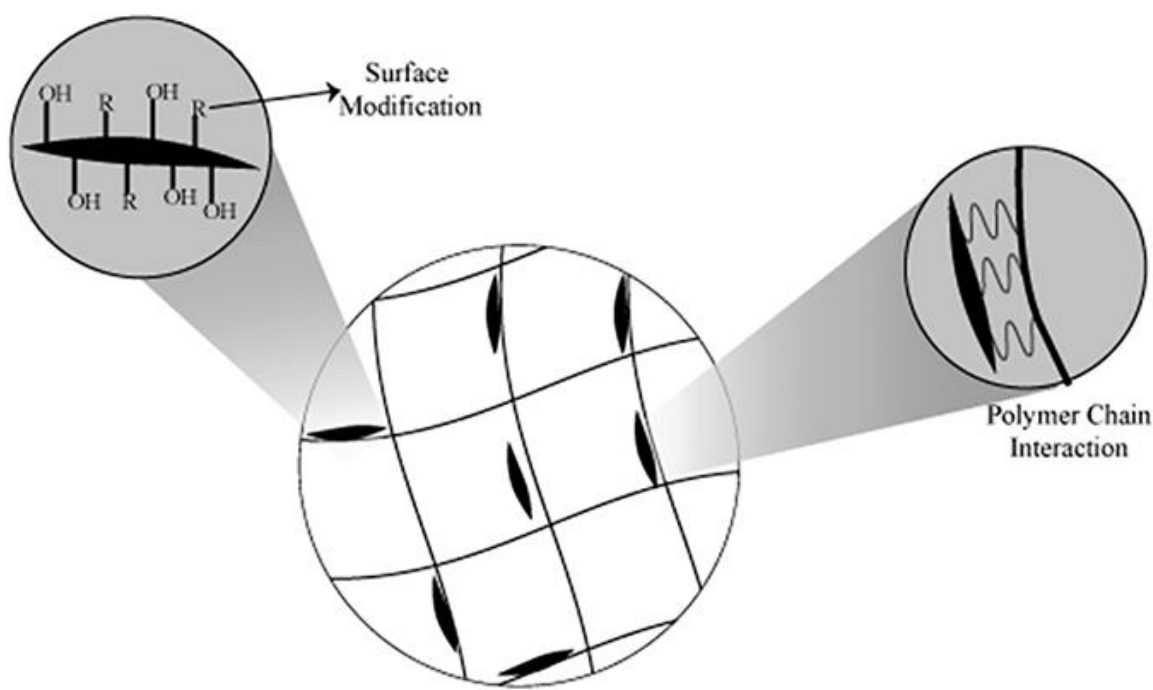


Figure 14.9 Cellulose nanocrystals have numerous hydroxyls on their surface, enabling them for functionalization or graphitization. This allows these materials to interact with specific segments of the polymer chains that form the gel network, giving mechanical reinforcement or other physical properties.

Vakili *et al.*⁵⁵ graphitized CNCs with poly(acrylic acid) improving the hydrogels rheological behavior in the presence of mucin, when compared to CNCs or other gels alone, due to the formation of so-called “hardcore bottle-brush polymer structures”. This material, complexed with cisplatin, presented a slow release of this drug, significantly increasing in inhibitory concentration in 50% of cells (IC₅₀) of the drug against HCT116 cells.

Karzar Jeddi and Mahkam⁵⁶ isolated CNCs through hydrolysis catalyzed by hydrochloric acid and functionalized their surface through carboxymethylation that is finally precipitated with Fe₃O₄ nanoparticles, making the CNCs magnetic. This material was incorporated into alginate hydrogels beads, giving them magnetic field responsive properties. These beads were tested as pH-sensitive in gastric (pH 1.2), intestinal (pH 7.4), and cancer cell fluid (pH 5.8) environments. The test with dexamethasone was shown to be the most sensitive for release at pH 5.8. Other works also show the functionalization of CNCs impregnated with iron oxides as additives to magnetic field responsive hydrogels.⁵⁷

Cationic surfactants such as CTAB (*N*-cetyl-*N,N,N*-trimethyl ammonium bromide) are frequently used to modify the surface of CNCs from hydrophilic to lipophilic.^{58–60} Gupta and Raghav⁶¹ compared the difference between CTAB and TBAB (tetra-*n*-butylammoniumbromide), another cationic surfactant, evaluating the release of non-steroidal anti-inflammatory drugs (NSAIDs). The authors conclude that both surfactants are able to alter the hydrophilicity of CNCs. However, the chain length of CTAB and TBAB impact the binding and release efficiency of drugs. CTAB, with a longer chain length, showed a higher binding efficiency and sustained drug release for a longer time. Natural surfactants, such as rarasaponin,⁶² can also be used for surface modification of CNCs to increase the adsorption of tetracycline compared to the unmodified CNC.

CNC surface functionalization can also make them a targeting ligand in chemotherapeutics. Seo *et al.*⁶³ used CNCs, with negatively-charged surfaces, to coat them with doxorubicin, and then this material was wrapped in hyaluronic acid, serving as a CD44 (tumor targeting) signal receptor mediating endocytosis. The adhesion of *cis*-aconityl-doxorubicin (CAD) on CNC does not change its dimensions, keeping its aspect ratio between 12 and 15, which facilitates endocytosis. In addition, CAD exhibits fluorescence which makes the CNC–CAD system conducive to fluorescence-visible drug delivery.

14.4.6 Bacterial Cellulose Hydrogel Membranes

BC is recognized for forming three-dimensional fibrous networks. With the versatility to also form empty cellulose packets, enabling drug storage,

introducing inulin into the fructose permeates of the *Gluconacetobacter xylinus* (ATCC 10245) bacterial strain.⁶⁴ Due to its biocompatibility BC can be applied directly to the skin and can also be combined with other additives, such as glycerol, for controlled delivery of glycolic acid,⁶⁵ beyond the possibility of incorporating growth factors (GFs) for duraplasty – a type of repair or expansion of dura mater, to treat traumatic brain injuries and spinal cord injuries.⁶⁶ BC also plays an important role in the production of scaffolds for bone regeneration.⁶⁷ Nano-fibrillated BC can also be combined with other cellulose derivatives, such as CMC or HPC, to carry hydrophobic anticancer agents such as paclitaxel.⁶⁸

There are also scale-up studies of BC production with *in situ* modification to increase pore size and accelerate drug release.⁶⁹ Commercial scale also requires higher BC productions and therefore some research studies aiming to optimize biosynthesis processes of this polysaccharide, through new strains such as *Komagataeibacter rhaeticus* TJPU03⁷⁰ or *Bacillus licheniformis* ZBT2.⁷¹

BC have been attracting widespread attention for drug delivery applications, since it is possible to form hydrogels from this material. Treesuppharat *et al.*⁷² inserted gelatin into the cavities of the BC networks, using glutaraldehyde as a crosslinker. Both materials exhibit compatibility and thermal stability, and the mechanical properties of the hydrogel were improved. The BC matrix can also be used for delivery implants of local drugs, as in chemotherapy. Cacicedo *et al.*⁷³ used BC to carry nanostructured lipids loaded with doxorubicin. The experimental evidence confirms that the hybrid hydrogel of the BC nanoparticle can be used in implants for local tumor treatment. The BC can also be incorporated into other gels, such as gelatin and carrageenan gum, decreasing the gelling time and improving the water uptake of these systems. These materials showed great viability and compatibility with cells, making them suitable for the formation of scaffolds, besides ensuring antibacterial properties, as shown in the Ngwabebhoh *et al.*⁷⁴ report.

14.4.7 Cellulose Derivative as Hydrogels

Other cellulose derivatives can also form drug delivery materials as HEC, able to form high viscosity systems at low concentrations and exhibit considerable elastic behavior. Such systems are interesting for viscous supplementation and can mimic the mechanical properties of fluids such as synovial fluid found in human joints.⁷⁵ Hydrogels containing CMC and MC modified with poly(methacrylate) have potential application for nucleus pulposus replacement as they are stable and non-toxic. Through a crosslink between the two cellulose derivatives the hydrogel can be gelled *in situ*, restoring the biomechanical properties of the nucleus pulposus.⁷⁶

CMC is also a cellulose derivative widely used to form hydrogels and can be combined with gelatin and crosslinked with glutaraldehyde,⁷⁷ and CMC nanogels can be formed with casein⁷⁸ or crosslinked with MCC.⁷⁹ Chen *et al.*⁸⁰ modified CMC with hydrazine and also oxidized it to different degrees of oxidation and through a Schiff base reaction formed copolymers with PEO-*b*-PDPA micelles, which are pH responsive and carry hydrophobic drugs. As a result, an injectable hydrogel is obtained, based on modified CMC. Cellulose triacetate (CTA) is capable of forming aerogels presenting its hydrophobicity as an advantage, which makes it more resistant to swelling and breaking when exposed in water. Wang and Okubayashi⁸¹ solubilized CTA in 1,4-dioxane at 70 °C and to this material was added a solution of ethanol with dissolved acetaminophen and after 5 h they obtained gels that were subsequently dried with srCO_2 to obtain aerogels.

Other cellulose derivatives, such as dialdehyde cellulose, can be used as crosslinkers for other gels, such as in chitosan gels containing zinc nanoparticles.⁸² They also have advantages such as low toxicity for the preparation of PVA hydrogel films.⁸³

14.5 Other Cellulose Materials for Drug Delivery Applications

14.5.1 Microcrystalline Cellulose

Another cellulose derivative that has been gaining importance is MCC, being one of the most used excipients in the pharmaceutical industry in various types of applications. New sources for obtaining this material are being explored. Pachau *et al.*,⁸⁴ for example, extracted MCC from the renewable biomass of *Ensete glaucum* with dilute acid hydrolysis. As a result, MCC with properties comparable to commercial standards was obtained.

MCC can be functionalized with silanized ionic liquids, making it pH-dependent and facilitating the adsorption of drugs such as Losartan.⁸⁵ There is also the possibility to solubilize MCC in ionic liquids and precipitate it in water, forming spherical beads that can be functionalized with iron oxides, providing them with magnetic field sensitivity.⁸⁶

Using MCC, it is possible to obtain materials with various morphologies. It is also possible to obtain self-floating tablets for metformin delivery.⁸⁷ In addition to spherical morphologies,⁸⁸ “brush-like” structures can be obtained. Bai *et al.*⁸⁹ synthesized cylindrical structures, with different sizes, by copolymers composed of MCC, poly(ethylene glycol) methyl ether methacrylate, as a hydrophilic block, and glutathione (GSH)-responsive hydrophobic camptothecin, as a drug for cancer treatments.

14.5.2 Cellulose Nanofibers

CNF can be utilized for scaffold fabrication, as presented by Kamel *et al.*⁹⁰ who used bleached bagasse to obtain CNF, subsequently oxidized with the catalyst TEMPO. This material served as the base for the scaffold formed together with cyclodextrins and raloxifene hydrochloride (selective estrogen receptor). The results showed that these scaffolds are highly porous and a controlled drug release pattern was achieved, and showed biocompatibility against the Saos-2 cell line, improved cell adhesion, and alkaline phosphatase enzyme expression.

Nanofibers can also form films and nanocomposites for drug delivery. Abukhadra *et al.*⁹¹ for example used exfoliated bentonite to form composites with CNFs for oxaliplatin delivery for colorectal cancer treatment.

14.5.3 Carboxymethyl Cellulose

CMC is commonly used for pharmaceutical purposes, presenting advantages as it is a soluble derivative. Nejabat *et al.*⁹² coated mesoporous silica structures, loaded with doxorubicin, with acetylated CMC and subsequently conjugated with AS1411 aptamer to guide drug delivery to nucleolin overexpressed cancerous cells. Copolymers with amphiphilic characteristics of polylactic acid with CMC can also be produced and can be used as hydrophobic drug carrier micelles. CMC fibers with poly(vinyl alcohol) are also used as a base for drug delivery of flufenamic acid through an electrospinning technique.⁹³

14.5.4 Hydroxymethylpropyl and Hydroxypropyl Cellulose

HPMC is also a widely used excipient in drug delivery. High molar mass HPMC can produce fibrous materials that expand in gastric fluid, preventing them from passing into the intestine, thus a slow delivery of acetaminophen was achieved. The authors reported that after immersion of this material in the gastric fluid the HPMC is able to expand up to 100% after 15 minutes, releasing slowly the drug. HPMC can also be functionalized, making it magnetic and pH-responsive,⁹⁴ mucoadhesive for oral delivery of ferrous sulfate⁹⁵ or mucoadhesive films for buprenorphine hydrochloride delivery.⁹⁶

Hydroxypropyl cellulose (HPC), in turn, is used in combination with other polymers to form floating tablets printed by hot extrusion.⁹⁷ But HPC can also be used for mucoadhesive oral films, as can HPMC.⁹⁸

14.6 Conclusions

Cellulose, the most abundant polysaccharide on planet earth, is an extremely versatile material for numerous applications, as in drug delivery. It stands out for having a structure that can be easily modified for numerous purposes, making it responsive to various stimuli, facilitating the release of the drug under specific conditions. The surface functionalization is also interesting because it allows the creation of target systems. In addition, nanocellulose has great prominence given its biocompatibility. Nanofibers are widely used to create three-dimensional networks, as well as bacterial cellulose, which are widely used to form gels and also aerogels, which are

gaining great notoriety. Cellulose derivatives are also prominent in drug delivery materials. Cellulose nanocrystals also stand out not only as reinforcing agents, but also as easily functionalized structures for drug delivery. In addition, biomaterials are replacing synthetic polymers, especially in the area of scaffolds and materials capable of replacing organic fluids, delivering greater compatibility in biological systems.

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Chapter 15

Starch-based Drug Delivery System: A Review on Pharmaceutical and Biomedical Applications

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15.1 Introduction

Starch, a plant-based natural polysaccharide produced by the assimilation of carbon towards the photosynthesis process in several plants and certain algal sources. The polymeric structure of starch was constructed by the multiple units of amylose and amylopectin joined together with glycosidic linkages. Different classifications of plant sources like potato, sweet potato, yam rice, and corn, *etc.*, consist of a maximum concentration of starch on their dry mass and few kinds of algal sources involved in photosynthesis also contain a specific concentration of starch.¹ Archeological reports and survey information reveals that humans of various habitats around the globe have adapted to starch-enriched foods in their diet since an ancient times.^{2,3} In addition, starch is an easily affordable and the most available carbohydrate for society as a regular diet and has health benefits.

The superior functional and physiochemical parameters of the starch were utilized in various industrial, biomedical, and pharmaceutical applications. In addition to being a dietary product, a starch base is widely used as a preserving, packing/baking, gelling, and stabilizing agent for the food materials in food industries.^{2,4} Furthermore, starch has also been used in other applications like manufacturing of paper, bioplastics, cosmetics, as an additive agent on textile fabrics^{5,6} and biofuel production.⁷ Another major application of starch is pharmaceutical where it acts as an absorbent, binding, and encapsulating agent.⁸ In recent decades, researchers have focused on starch-based micro- and nano-immobilization techniques which are widely utilized as advanced DDS for the treatment of different kinds of physiological disorders.

The advanced therapeutic system evolved on the fabrication of targeted DDS in which the therapeutic agents were loaded into the micro- and nano-sized carrier systems. The ultimate purpose of fabricating the micro- and nano-carriers for pharmacological application is to release the therapeutic agents at desired physiochemical parameters within the living organisms. The physiochemical parameters include pH, temperature, pressure, surface area, conductivity, and solubility, which varies based on the affected/infected regions of the living organisms. Most of the DDSs were designed to digest at desired physiochemical phenomenon and release the therapeutic agents. Therefore, the targeted release of the therapeutics from the micro- and nano-carriers significantly increases the concentration of the therapeutics on biological fluids in humans *i.e.*, bioavailability. The fabrication of DDSs was carried out with different classifications of metal, metal oxides, and polymeric nanoparticles (NPs) to evaluate its potential as a therapeutic agent for diverse biological and pharmaceutical applications.^{9,10}

Extensive evaluation of metal and metal oxide-based DDSs under *in vitro* and *in vivo* model systems resulted in adverse toxic effects on living organisms. The metal and metal oxide sources were the reasonable factors for reactive oxygen species (ROS)-mediated oxidative stress (OS) inducing DNA damage, lipid peroxidation, and protein oxidation, causing cellular damage, ultimately leading to organ disorders in the animal system. Development of polymer-based DDSs helped to overcome the toxic effects

of metal and metal oxide-based DDSs in living organisms. The advantage of polymers as a DDS includes low toxicity, excellence in loading and releasing of the therapeutics, and appropriate stability and solubility for the controlled release of therapeutics.¹¹ Among the different types of polymers sources, natural polymers such as cellulose, alginate, agar, carrageenan, and starch were accounted for as an excellent DDS without any harmful effects on humanities.¹² Starch exhibited an outstanding performance as a DDS due to its low-toxicity, low cost, ready accessibility, biocompatibility, and mostly applicable for functional and structural modification to become a wide variety of micro- and nano-DDS.¹³ This chapter focuses on essential sources of starch, different approaches for the fabrication of starch as a micro- and nano-DDS, surface modifications, and its potential applications as a therapeutic agent. Wet grinding, sieving, and drying are major ways of extracting and refining starch in the industry. It can be utilized in the natural form after extraction from plant “native starch” or undergoing one or more modifications (physical, mechanical, and/or chemical) to achieve targeted properties and become “modified starch”.

15.2 Major Composition of Starch and its Sources

Starch is a homopolymer composed of repeating units of alpha glucopyranose with the chemical formula $(C_6H_{10}O_5)_n$. Rich sources of starch include cereals, grains like wheat, barley, rice, and corn, seeds of legumes like peas and pulses, tuberous sources such as potato, sweet potato, ginger, turmeric, and groundnut, and immature fruits and vegetables. Based on the source, starch differs in granular size, particle morphology, and proportion of amylose and amylopectin. Starch is composed of two polymer chains – amylose and amylopectin. Amylose is an unbranched linear chain composed of 600 glucose units linked together by α 1–4-d-glycosidic bonds possessing a helical structure and is water soluble in nature. Amylopectin is a branched structure made of more than 50 000 glucose units linked by α 1–4 glycosidic bonds and branched at every 25–30 glucose units joined to the main chain by a α -(1 $\rightarrow$ 6) glycosidic bond. Branching in amylopectin prevents helical structure formation leading to creation of a spongy reticular

structure.^{13,14} Almost all the plant extracted starch consists of $20 \pm 5\%$ of amylose, $80 \pm 5\%$ of amylopectin, and a traceable percentage of protein, lipid, and phosphate on their granular mass,¹⁵ while the starch from genetically modified maize, barley, rice, and potato contained up to $100 \pm 1\%$ of amylopectin on their mass. Ratio of amylose and amylopectin determines the crystallinity, granular size, chemical nature, and polymer arrangement which influences the pore size of starch nanoparticles. Table 15.1 was collectively reported based on previous reviews, which displays the percentage of amylose and amylopectin present in different plant sources including yam, potato, sweet potato, tapioca, and various cereals like wheat, rice, maize, barley and others.^{14,16,18}

Table 15.1 Percentage of amylose and amylopectin in various plant sources of starch extraction.

S. no.	Plant source	Amylose (%)	Amylopectin (%)	Ref.
1	Maize	25	75	14
2	Wheat	23	77	
3	Rice	19	81	
4	Potato	22	78	16
5	Tapioca	17	83	
6	Banana	20	80	
7	Barley	27.5	72.5	
8	Arrowroot	25.6	74.4	
9	Lotus root	15.9	84.1	
10	Lentil	29–45	71–54	18
11	Yam	22	78	
12	Sweet Potato	18.9	81.1	
13	Chestnut	19.6	80.4	
14	Smooth pea	41.5 ± 8.5	± 8.5	14, 16–18
15	Wrinkled pea	74.5 ± 13.5	± 13.5	
16	Genetically modified starch from cereal sources	< 1	100 ± 1	

15.3 Methods Used in the Fabrication of Starch-based Micro/nano DDSs

In recent decades, DDSs have utilized the micro- and nano-sized materials for the immobilization of therapeutic agents like medicines, drugs, nutrients, and enzymes. In general, the term immobilization denotes an arrest or control of mobility, and similarly, the micro- and nano-immobilization techniques implicate the therapeutic agents to be combined with micro- and nano-scaled materials for the controlled release or flow of the therapeutics in the biological system.^{19–21} As such, starch-based micro- and nano-immobilization techniques utilized starch as a carrier system for the conjugation of therapeutic agents using diverse fabrication techniques and some of these approaches are discussed below.

15.3.1 Graft Copolymerization

Graft copolymerization is about grafting the monomer units from the branched polymer structure.²² Starch-based graft copolymerization for the fabrication of DDSs involves the grafting of amylose and amylopectin units into several nanometer-sized particles from the gelatinized source of starch incorporated with therapeutic agents. Whereas, the gelatinization of starch is the process of breaking the binding structure of the polymer system by treating the starch source at 80 °C under aqueous conditions. Then the therapeutic agents were incorporated into the gelatinized source of the starch through physical and chemical binding factors. At the end process of drying at 80 °C up to 12 h, the gelatinized starch loaded with therapeutics such as phloroglucinol and methyl gallate were grafted into nano DDSs by the kinetics of retrogradation.^{23,24}

15.3.2 Microemulsion

When the hydrophobic and hydrophilic phases are added together they neither immerse nor disperse. In the case of microemulsion, hydrophobic and hydrophilic phases were combined along with the addition of surfactants or cosurfactants, which leads to the immersion of one phase with another one. Here, the solubility of the hydrophobic phase through the

surfactant or cosurfactant tends to disperse the droplets of either a hydrophobe or hydrophile within another liquid; where the droplet size was observed to be ≤ 100 nm for microemulsions and >100 nm for macroemulsions respectively.²⁵ A microemulsion system for the production of NPs was carried out by two types of emulsions based on their dispersion such as oil-in-water emulsion and water-in-oil emulsion.²⁶ Most of the previous reports on microemulsion systems for the fabrication of starch-based DDSs were done with a water-in-oil microemulsion approach. The combination of water-in-oil emulsion made up of starch dissolved in a hydrophilic phase and hydrophobic phase mixed by the addition of emulsifying agents (such as span 60, tween 80), organic solvents, and ionic liquids as a surfactant and cosurfactant for the fabrication of starch-based DDSs by emulsion-based precipitation. The water-in-oil microemulsion approach on starch was obtained with the yield of micro- and nano-sized carriers loaded with therapeutics like curcumin and indomethacin as a starch-based DDS.^{27–29}

15.3.3 Ultrasonication

Ultrasonication is a kind of top-down approach for the conversion of macromolecules into several nanometers to micrometer scale. Ultrasonication waves are sound waves with a higher frequency (>16 kHz) than the audible frequency of human beings.³⁰ Typically, starch-based micro- and nano-DDS was produced by the treatment of the aqueous or gelatinized suspensions of the starch along with therapeutics under the ultrasonication frequencies. The cavitation phenomenon formed during the treatment of starch suspension with ultrasonic waves at around 20–50 kHz leads to particle size reduction in order to produce the DDS on the scale of several nanometers to micrometers. Starch-based DDS produced using ultrasonic waves results in variation in their morphology and size based on their sonication period. Ultrasonication approaches for the fabrication of starch-based DDS retained better loading and releasing kinetics on certain therapeutics such as peppermint oil, zolpidem, and resveratrol.^{31–33}

15.3.4 Hydrolysis

Hydrolysis of starch was performed by breaking the bond on the starch polymeric structure with the splitting of water into hydrogen and hydroxyl parts leading to the formation of micro- and nano-sized materials. The chemical route of starch hydrolysis was carried out with acidic sources like sulfuric acid and hydrochloric acid. On the other hand, the biochemical route of starch hydrolysis was carried with bio-extracted enzymes like α -amylase, β -amylase, pancreatin, and glucoamylases. Both routes employed the role of hydrolysis for the fabrication of starch-based micro- and nano-carriers for the loading therapeutic and organic agents.^{34–37}

15.3.5 Electrospinning

Electrospinning is a voltage-directed process that produces the ultra-fine nano- and micro-scale fibers of polymers through the electrohydrodynamic phenomenon. Consequently, the electrospinning process for the fabrication of starch-based DDSs was assessed with the native starch, modified starch, and also the combination of non-starchy polymer derivatives.³⁸ The working setup of the electrospinning consists of a needled syringe with a control pump, electric power at high voltage, and the collecting unit on the ground state. In detail, the syringe part loaded with polymeric suspension was pumped to eject the droplet of polymer and the droplet was significantly turned into a Taylor cone due to the higher electrostatic charge. Thereafter, the solvent phase present in the polymeric suspension evaporates and it gets deposited in the ground collector state as nano- or micro-sized fibers. The electrohydrodynamic phenomenon of electrospinning was previously assessed for the fabrication of micro- and nano-DDS using the starch derivatives and the starch combined composite materials for the encapsulation of therapeutic agents including diclofenac, ampicillin, and chlorpheniramine maleate.^{39–42}

15.4 Significance of Starch as a Drug Delivery System

The standard aspects of DDSs have been illustrated according to the research survey of recent decades. Consequently, the advanced DDS should obey the following criteria such as (i) highest loading and sustained release

of the therapeutic agents, (ii) permeability on biological barriers such as cell membranes and blood brain barriers (BBB), (iii) appropriate stability and digestion at the desired parameter, (iv) biocompatibility, and (v) biodegradability on living mammals.⁴³ As such, starch-based DDS withstands the entire above phenomenon with its superior properties and behaviors. The superior flexibilities of starch like gelatinization and retrogradation were the reasonable factors that influence the native starch for the different classes of physical, chemical, and biochemical modifications. The physical modification on starch meets the drying, annealing, lyophilization, ultrasonication, mechanical milling, high pressure, and electrical influences for the transformation into DDSs. The chemical modification of starch into DDSs involves a chemical kinetic process such as oxidation, esterification, cross-linking, polymerization, acetylation, and hydrolysis. Biochemical modification is about the treatment of starch with bio-extracted enzymes like α -amylase and β -amylase which modifies the starch to a suitable DDS.^{4,44,45} The above modification in starch enhances the potential efficiency such as drug loading capacity, swelling, and solubility on biological fluids in order to release the therapeutics in a controlled manner. Besides the above, starch and its derivatives were degraded by the mammalian enzymes and exhibit no or less toxic effects on human beings and animals which confirms the biodegradability and biocompatibility of the starch as a DDS.¹³ Previous reports on starch-based DDS ensure its permeability on biological membranes which denotes the possibilities of starch-based DDS to transverse the cell membrane, BBB, vascular region, and other biological fluids.^{23,24} Figure 15.1 illustrates the major plant sources for the bulk extraction of native starch towards the methods of fabricating the starch-based DDS and their application in various fields.

Starch Enriched Plant Sources

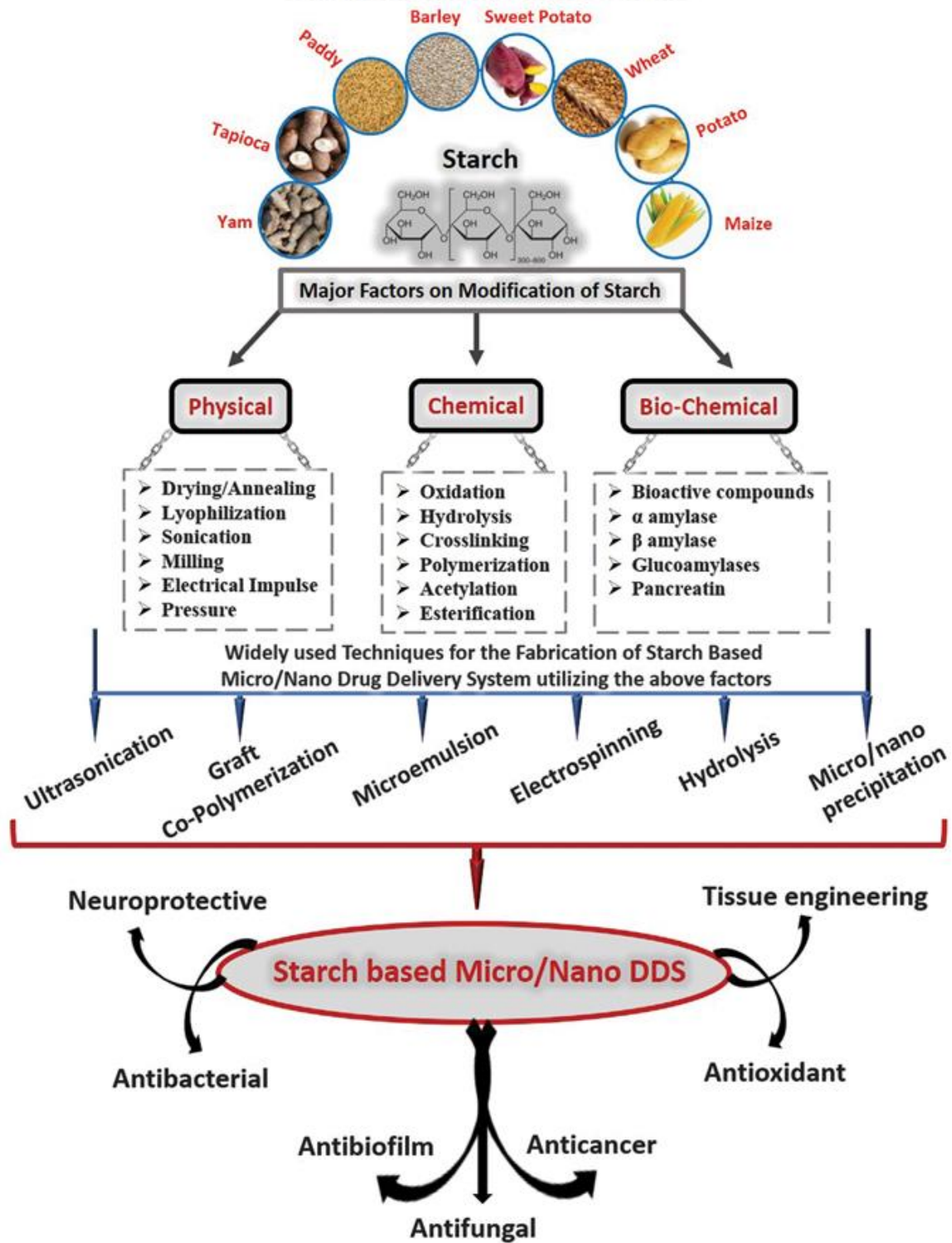


Figure 15.1 Understanding of various plant sources for starch extraction with the physical, chemical, and modifications of starch in order to produce starch-based micro/nano DDS.

15.5 Limitations in Starch and Starch-based DDSs

Although starch-based polymer systems have some limitations, which are almost applicable for the several kinds of natural polymers. The common limitations of the natural polymers observed in starch include (i) complication and long-term processing of its extraction from the natural sources, (ii) variation in its composition based on the plant source and period of isolation, (iii) production of the starch towards the yield of starchy plants are seasonal, (iv) possibilities of microbial contamination, and (v) its moisture sensitive behavior.^{12,43} Additionally, the moisture absorption behavior of the starch affects its flow, binding affinity, and tensile strength based on the composition of DDS.^{46,47} With reference to our previous studies the phase transition due to the retrogradation significantly affects the crystalline structure of the starch. Another major obstacle in nanosized starch-based DDS is agglomeration of starch nanoparticles which affects the cellular penetration of starch-based nanoparticles reducing its biomedical efficiency, specifically its antimicrobial potential.^{24,48} Hence, the above-described factors were considered as an exceptional limitation of starch in the fabrication and functionalization of the DDSs.

15.6 Application of Starch-based DDSs

Starch-based micro- and nano-sized materials are highly applicable for biomedical, food, cosmetics, agriculture and other biological applications. The starch-based DDS with increased loading and sustained release of the therapeutics showed excellent pharmaceutical potential as an antimicrobial, anticarcinogenic, antioxidant, and neuroprotective agent and also in bone and tissue engineering applications.^{47,49,50} Table 15.2 describes the various kinds of starch-based DDS and their applications. Major steps involved in starch-based DDS were (i) the encapsulation of therapeutic agents within the starch micro/nano-carriers and (ii) the combination of the starch with

the micro/nano composites to become a therapeutic agent or else the micro/nano composite materials were incorporated with the therapeutic agents. The antimicrobial activities of the starch-based micro/nano DDS showed their efficiency against certain kinds of bacterial strains and fungal spores and starch-based micro/nano DDS also exhibited inhibition on biofilm produced by the bacterial strains.^{48,51–53} In the case of anticarcinogenic applications of starch-based DDS treated with different types of cancer cells they resulted in improved anticancer effects with less toxic effects.^{54–56} Efficient conjugation and delivery of therapeutic agents like vitamins, phytochemicals, and ayurvedic compounds exhibit excellent antioxidant behavior significantly and were useful for various health benefits such as neuroprotective applications.^{23,24,48,57} An immobilization of therapeutic agents with starch-based micro/nano materials ensures the protection of the therapeutic agents from physiological factors like oxygen, pressure, temperature, and light. Therefore, starch-based immobilization strategies were useful for various therapeutic applications toward the increased bioavailability of the drugs in humans.⁵⁸

Table 15.2 Fabrication techniques for starch-based DDS and their applications.

S. no.	Fabrication technique	Starch source	Micro/nano DDS	Applications	Ref.
1	Graft copolymerization	N/A	Starch nanoparticles loaded with phloroglucinol	Anticancer effect on HepG2 cell line	²³
2		Maize starch	Triphala churna loaded starch nanoparticles	Antibacterial, antibiofilm, and antioxidant applications	²⁴
3			Starch encapsulated	Neuroprotective potential on A β	⁴⁸

4	Microemulsion	Sago starch	methyl gallate Curcumin loaded starch nanoparticles	peptide and Neuro2A cells Evaluation of drug loading and releasing kinetics useful for the futuristic applications	27
5		Maize starch	Indomethacin conjugated starch nanoparticles		29
6			Penicillin and streptomycin loaded starch nanoparticles	Antibacterial effect on <i>Streptococcus pyogenes</i> bacterial strain	59
7			Flufenamic acid, testosterone, and caffeine loaded starch nanoparticles	Determination of the permeability of nanoencapsulated drugs on human skin	75
8	Ultrasonication	Chestnut starch	Starch nanocapsules loaded with resveratrol	Anti-diabetic and anti-obesity applications	33

9	Nanoprecipitation	Maize starch	Diclofenac sodium loaded starch nanoparticles	Histological studies on female rats	60
10			Paclitaxel loaded pores starch nanoparticles	Anticancer assessment on mouse (LLC) lung cancer cells	61
11		Banana starch	Starch nanoparticles with loaded curcumin	Evaluation of curcumin release on simulated gastric and intestinal fluids	63
12		Potato, pea, and corn starches	Quercetin incorporated starch nanoparticles	Determination of the antioxidant effect with a DPPH assay	67
13		N/A	Curcumin loaded starch nanoparticles	Antibiofilm evaluation on <i>Streptococcus mutans</i> bacterial biofilm	79
14		Maize starch	Ciprofloxacin loaded acetylated starch nanoparticles	N/A	81
15		Sago starch	Paracetamol loaded starch–citrate nanoparticles	Cytotoxicity evaluation on human keratinocyte (HaCaT) cells	84

16	Hydrolysis	Potato starch	Starch nanoparticles	Assessment of the inflammatory response of starch nanoparticles on human THP-1 monocytic cells	64
17		Rice starch	Doxorubicin loaded acetylated starch nanoparticles	Anticarcinogenic effect on HeLa human cervical cancer (CCL-2) cells	66
18		Maize starch	Combination of starch nanocrystals and gum arabic blends with loaded hydroxyurea	Evaluation of the anticancer effect toward human colon cancer (LS-174) cells	71
19		Cassava starch	Curcumin loaded starch nanoparticles	Anticancer potential on human malignant melanoma (SKMEL-28) cells	86
20	Combination of hydrolysis and ultrasonication	Maize starch	Fingolimod (FTY720) loaded starch nanoparticles	Evaluation of the anticancer effect on human breast cancer (MCF-7) cells	73
21	Combination of hydrolysis and precipitation	Potato starch	Paclitaxel loaded starch nanoparticles	Cytotoxicity evaluation on mouse bone marrow (7F2) cells	37

22	Double-emulsion	Potato starch	Calcein loaded starch acetate microparticles	Evaluation of cells on the viability of starch microparticles on retinal pigment epithelium (RPE) cells	62
23		Rice starch	Metronidazole loaded starch microsphere	Assessment of the antibacterial effect on <i>Bacteroides fragilis</i>	83
24	Sacrificial hard-template	Pea, mung bean, potato, and maize starches	Doxorubicin loaded hollow starch nanoparticles	Cytotoxicity evaluation on alpha mouse liver 12 (AML 12) and human liver cancer (HepG2) cells	65
25	Dialysis	N/A	Curcumin-loaded starch nanoparticles	Assessment of anticancer effect toward human lung cancer (A549) cells	68
26	Emulsion-solvent diffusion	Maize starch	CG-1521 (7-phenyl-2,4,6-hepta-trienoyl hydroxamic acid) loaded starch nanoparticles	Evaluation of anticancer potential on human breast cancer (MCF-7) cells	69

27	Self-assembly	N/A	Chloroquine conjugated starch nanoparticles	Anticancer evaluation on pancreatic cancer (Mia Paca-1, Mia Paca-2 and AsPC-1) cells	70
28			Insulin loaded PEGylated starch acetate nanoparticles	Toxicity evaluation on mouse fibroblast (L929) cells	77
29			Morin conjugated hydroxyethyl starch-deoxycholic acid nanoparticles	Evaluation of morin release on <i>in vitro</i> and <i>in vivo</i> models	80
30	High-speed shearing emulsification	N/A	5-fluorouracil loaded starch nanoparticles	Evaluation of 5-fluorouracil release on simulated colonic fluid	72
31	Electrostatic layer by layer deposition	Cassava starch	Bovine serum albumin loaded starch nanocapsules	Evaluation of bovine serum albumin on simulated gastric, intestinal, and colonic fluids	76

32	Cross linking	Potato starch	Insulin incorporated starch nanoparticles	Evaluation of insulin release on <i>in vitro</i> and <i>in vivo</i> models	78
33		N/A	Ciprofloxacin loaded starch nanoparticles	Evaluation of drug release	82
34		Jackfruit seed starch	Ibuprofen loaded starch microspheres	Evaluation of ibuprofen release at different pH	85
35	High pressure homogenization	Maize starch	Peppermint oil–starch nano emulsions	Assessment of antibacterial effect	74

15.6.1 Antimicrobial Evaluations of Starch-based DDSs

The potential of starch-based DDSs as antimicrobial agents was evaluated against various fungal and bacterial strains. Akturk *et al.* evaluated the antifungal property of starch-based DDS encapsulating copper NPs against *Candida albicans* and *Candida krusei*. The results revealed that starch-stabilized metal NPs showed a higher antifungal effect when compared to other polymer-stabilized metal NPs. The soluble starch-capped metal NPs exhibited increased stability against aggregation and other disturbing environmental factors.⁵³ Ismail *et al.* (2016) fabricated the single or multiple antibiotic encapsulated starch NPs by the microemulsion method, which was observed to be highly stable and reported for its potent antimicrobial activity against *Streptococcus pyogenes* and *Escherichia coli* under alkaline conditions.⁵⁹ Ramnath *et al.* reported the synthesis of colloidal silver nanoparticles (Ag NPs) using cow pea starch in which the hydroxyl groups in the aldehyde terminal reduced silver nitrate (AgNO₃) to silver metal, simultaneously stabilizing the resulting AgNPs. Hydroxyl groups of starch were responsible for the shape, size, and dispersion tunability of Ag NPs enhancing its biodegradability and biocompatibility. Antimicrobial assessment revealed that starch-stabilized Ag NPs exhibited bactericidal activity against Gram positive and negative bacterial strains at

the minimum inhibitory concentration.⁵⁶ Prakashkumar *et al.* reported the antimicrobial and antibiofilm activities of starch-encapsulated triphala churna synthesized by a graft polymerization method. Results of the study showed enhanced antibacterial effects against *E. coli*, *Shigella dysenteriae*, *Salmonella typhi*, and *Staphylococcus aureus* bacterial strains at its lowest concentration when compared to drug alone, which might be due to penetration of nanosized drug through the bacterial cell membrane and release of the drug at the desired site. Starch-encapsulated triphala extract showed potent antibiofilm activity against methicillin resistant *S. aureus* by inhibiting cell to cell contact, detaching the biofilm exhibiting disrupted biofilm architecture with microcolonies.⁵⁹ Cross-linked blend films of polyvinyl alcohol (PVA) and quaternary ammonium starch (ST-GTMAC) using citric acid (CA) as a plasticizer and glutaraldehyde (GA) as a cross-linker showed antimicrobial properties against Gram positive (*S. aureus* and *Bacillus subtilis*) and Gram negative (*E. coli* and *Pseudomonas aeruginosa*) bacteria.⁸⁷ Starch-based DDS have also been investigated for the fabrication of films and composite materials made up of micro- and nano-sized starch substrates for the loading of essential oil, drugs, and other organic compounds as a therapeutic agent. Liang *et al.*, synthesized the peppermint oil incorporated starch – MCT (medium-chain triacylglycerol) nanoemulsions which retain the antibacterial effect against *Listeria monocytogenes* and *S. aureus* bacterial strains. Results of the study revealed that the peppermint oil loaded with starch – MCT nanoemulsions exhibited long-term antibacterial effects on food pathogens when compared to the peppermint oil alone, which denotes the importance of the nanoencapsulation of peppermint oil in food packaging applications.⁷⁴ The multiple health benefits and naturally derivable aspects of essential oils like cinnamon, peppermint, oregano, lime, and grape seed when incorporated with starch-based DDS improve its efficiency as an antimicrobial agent, in food processing, and packaging applications.^{88,89} Starch-based micro/nano DDS due to its natural origin, ease of availability, biocompatibility, biodegradability, permeability, hydrophilicity, non-toxicity, non-immunogenicity, surface modification ability, bioadhesion property, and better drug carrying capacity have been widely used as antimicrobial agent-loaded nanocarriers. Wide antimicrobial applications of starch-based

micro/nano DDS make it useful for various biological, food processing, and pharmaceutical applications as starch NPs act as barriers against infectious and environmental microorganisms.

15.6.2 Antioxidant Potential of Starch-based DDSs

Generally, phenolic compounds and their derivatives from herbs, fruits, vegetables, and floral sources are highly enriched with antioxidants. Production and accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) induces lipid peroxidation, protein oxidation, and DNA damage, impairing the normal cellular function ultimately leading to organ dysfunction. Natural antioxidants like minerals, vitamins, enzymes, and polyphenols widely distributed in fruits and vegetables which significantly neutralize the ROS and RNS^{90,91} are useful for the treatment of oxidative stress-mediated disorders such as Alzheimer's, rheumatoid arthritis, cancer, cardiovascular disease, and chronic inflammation.^{92,93} Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and esters of gallic acid were also used as antioxidants. However, these natural and synthetic antioxidants possess certain limitations such as insolubility, low stability, poor bioavailability, and short half-life period. Recent progression in nanotechnology revealed that several inorganic/biological origin nanoparticles possess antioxidant properties. Encapsulation and entrapment of natural antioxidants in biodegradable nanogels and nanospheres improved the bioavailability and efficiency of natural antioxidants with reduced volatility and leaching. Recently, starch-based DDSs have received much attention for the delivery of antioxidants as it helps to overcome limitations such as poor oral bioavailability, insolubility, instability, and low intestinal absorption of antioxidants. In addition, the synergistic antioxidant potential of starch nanoparticles and loaded antioxidant improves the antioxidant efficiency.

Farrag *et al.* fabricated quercetin-loaded starch NPs using maize, potato, and pea starches through a nanoprecipitation approach and assessed its antioxidant potential by free radical scavenging assay. Results revealed that the potato starch-based nanoencapsulated quercetin showed higher free radical scavenging activity when compared to maize and pea starch nanoencapsulates which might be due to higher loading and controlled

release of quercetin by potato starch.⁶⁷ Kumar *et al.*, demonstrated the antioxidant evaluations of phloroglucinol loaded starch NPs synthesized using a graft copolymerization technique. Upon controlled release of phloroglucinol, the antioxidant exhibited potent DPPH, ABTS⁺ free radical scavenging, and minimal reduction power with respect to the electron transferring ability of nanoencapsulated phloroglucinol.²³ Prakashkumar *et al.*, reported immobilization of triphala churna and 7-methyl gallate with starch NPs using a graft copolymerization approach. Nanoencapsulated triphala churna and methyl gallate showed enhanced free radical scavenging activity and reducing power when compared to antioxidant alone which might be due to the synergistic effect of starch nanoparticles with loaded antioxidants. These antioxidant-rich nanoencapsulated drugs also showed potent acetylcholinesterase (AChE) inhibitory activity under *in vitro* conditions^{24,48} which makes them beneficial for the treatment of Alzheimer's disease and other neuronal disorders. In addition, starch-encapsulated methyl gallate also prevented the aggregation of amyloid beta (A β) peptides and disaggregated the preformed fibrils which play a vital role in AD pathogenesis. Furthermore, the size of starch encapsulated methyl gallate is less than 500 nm which facilitates penetration into the blood brain barrier, depicting the fact that starch nanoparticles as a suitable DDS for Alzheimer's therapy.²⁴ Ahmad and Gani demonstrated the fabrication of resveratrol incorporated starch nanocapsules and assessed their antioxidant behavior. The antioxidant behavior of the resveratrol loaded on starch nanocapsules was evaluated based on the inhibitory rate of enzymes α -glucosidase, cholesterol esterase, and pancreatic lipase the causatives of obesity and diabetes. The nanoencapsulated resveratrol shows enhanced anti-obesity and anti-diabetic effects when compared to resveratrol alone.³³ Polyphenols like (+)-catechin, (-)-epicatechin, (-)-epigallocatechin-3-gallate, and proanthocyanidins, were loaded with starch nanoparticles (SNPs) and assessed for their antioxidant potential under different conditions of ionic strength, high temperature, and ultraviolet radiation. Monodisperse spherical shaped polyphenol-loaded SNPs (PSNP) were obtained with no agglomeration and restored the antioxidant potential of polyphenols at different pH, temperature, and UV irradiation. PSNPs showed non-cytotoxic effects in mouse embryonic fibroblast cells

illustrating their biocompatibility and proving the fact that starch can act as an effective nanocarrier for protecting bioactive compounds against sensitive environments and to control their release.⁹⁷ Piroxicam-loaded starch nanoparticles prepared by a nanoprecipitation method showed enhanced solubility and antioxidant activity and 98.8% release of drugs within 2 h at physiological pH.⁹⁸ Thus, the synergistic effect of antioxidants incorporated on starch-based micro/nano DDS may be useful for the treatment and recovery of various ROS/RNS-based physiological disorders.

15.6.3 Cellular Uptake, Cellular Viability, and Cytotoxic Effects of Starch-based DDSs

The prediction of cell viability, cell mediated toxicity, and cellular uptake of starch-based micro/nano DDSs were typically essential for the therapeutic progress of various physiological disorders. Basically, these disorders are caused by infectious, non-infectious, hereditary, and injury-based factors. Thereby, the cell viability, cellular uptake, and cytotoxicity profiles of therapeutic agents have to be observed for the toxicological and pharmacological findings on living organisms.⁹⁴ The cell viability and cytotoxicity profiles of starch-based micro/nano DDSs were determined by *in vitro* assay system which consists of the cells extracted or genetically modified from the normal, physically affected or the infectious tissues or organs of human beings and animals. The above *in vitro* assessments on starch-based micro/nano DDS were used to assess the anticancer potential of anticancer therapeutic agents conjugated with starch NPs. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is the widely used *in vitro* assay system for the evaluation of cytotoxicity and anticancer effect of starch-based micro/nano DDSs.^{95,96} HepG2 human liver cancer cells and AML12 liver cells from transgenic mouse were used to assess the cytotoxic effects of therapeutics loaded starch-based DDS.^{23,65} Phloroglucinol loaded starch nanoparticles showed cytotoxic effects against HepG2 cells.²³ Yang *et al.*, fabricated the hollow starch nanoparticles incorporated with doxorubicin which showed more viability on AML liver extracted cells when compared to HepG2 liver cancer cells revealing the fact that doxorubicin loaded hollow starch NPs exhibit cytotoxicity toward liver cancer cells and are biocompatible with normal liver cells.⁶⁵ The

anticancer potential of starch-based DDS for lung carcinoma was assessed using lunon LLC (Lewis lung carcinoma) and A549 cancer cells extracted from mouse and human lung cancer cells.^{61,68} Wang *et al.*, reported the cytotoxic effect of paclitaxel loaded porous starch NPs fabricated *via* a nanoprecipitation approach using CCK-8 (Cell Counting Kit-8) to assess the cell viability. Paclitaxel loaded porous starch nanoparticles exhibit an enhanced cytotoxic effect toward LLC lung cancer cells when compared to paclitaxel alone.⁶¹ Xu *et al.*, pointed out that the curcumin loaded starch nanoparticles showed an enhanced cellular uptake and anticancer effect in A549 human lung carcinoma cells. Additionally, the curcumin loaded starch nanoparticles also showed better hemocompatibility in rabbit's red blood cells revealing its biocompatible nature.⁶⁸

Anticancer potential of starch-based DDSs in breast cancer was evaluated using MCF-7 human breast cancer cells as the model system.^{69,73} Alp *et al.* fabricated the CG-1521 loaded starch NPs by a solvent diffusion approach and examined its anticancer benefit and release kinetics of CG-1521. CG-1521 (7-phenyl-2,4,6-hepta-trienoyl hydroxamic acid) normally inhibits histone acetyltransferase and histone deacetylase (HDAC) which are the major causatives of various carcinoma. Nanoencapsulated CG-1521 exhibited anticancer potential in MCF-7 cells by inhibiting the proliferative factors causing cancer.⁶⁹ On the other hand Masoudipour *et al.* reported the enhanced anticancer potential of fingolimod (FTY720) loaded starch NPs against MCF-7 human breast cancer cells. FTY720 is an FDA approved immunosuppressor widely utilized for cancer therapy. Starch loaded FTY720 released the drug in a slow and sustained manner enhancing the antiproliferative effect in MCF-7 cells when compared to the FTY720 alone.⁷³

Starch-based DDS fabricated based on the hydrolysis techniques produced doxorubicin, hydroxyurea, and curcumin incorporated starch NPs.^{66,71,86} Xiao *et al.*, synthesized doxorubicin loaded acetylated nanocrystals which showed enhanced cellular uptake and anticarcinogenic effects toward HeLa human cervical cancer cells. The anticancer potential of doxorubicin was observed to be high while it was loaded with acetylated starch nanocrystals when compared to non-acetylated starch nanocrystals and drugs alone.⁶⁶ Alwaan *et al.* fabricated hydroxyurea loaded blends

which consist of starch nanocrystals and Arabic gum exhibiting better anticancer effect on LS-174 human colon carcinoma cells.⁷¹ Geetha *et al.* reported the fabrication curcumin loaded starch–PVA (poly(vinyl alcohol)) nanocomposite and evaluated its cytotoxic effect in L929 mouse fibroblast cells and SKMEL-28 human malignant melanoma cells. The results revealed that curcumin loaded starch–PVA nanocomposites showed no cytotoxic effect toward L929 mouse fibroblast cells *i.e.*, normal cells and moderate cytotoxicity toward SKMEL-28 malignant melanoma cells⁸⁶ revealing its biocompatible nature. Sleightholm *et al.* investigated the chloroquine conjugated starch NPs for the anticancer evaluations on Mia Paca-1, Mia Paca-2, and AsPC-1 pancreatic cancer cells. In this case, the sustained release of the chloroquine results in a better anticancer effect on the above pancreatic cancer cells.⁷⁰ The starch NP-based DDS promotes an enhanced anticancer effect due to its cell permeability, and controlled release of the therapeutics with respect to the pH of carcinoma cells and nontoxic/non-irritant effect toward normal living cells. Based on the above-mentioned factors, starch-based DDS showed potent anticancer potential in 7F2 mouse bone marrow cells,³⁷ human THP-1 monocytic cells,⁶⁴ L929 mouse fibroblast cells,⁷⁷ and HaCaT human keratinocyte cells⁸⁴ proving the fact that a starch nanocarrier can act as a promising DDS for cancer therapy.

15.6.4 Drug Loading and Releasing Assessment of Starch-based DDSs as a Futuristic Prospect

Drug loading and drug releasing kinetics are very essential to illustrate the passage and bioavailability of the therapeutic agents loaded on DDS. Likewise, almost all the starch-based DDS have been examined for the concentration of the therapeutic agent loaded and released from the carrier system. Generally, loading of therapeutic agents within the starch micro/nano DDS was accomplished by either *in situ* and *ex situ* incorporation; which means the therapeutic agents were incorporated during the synthesis of micro/nano-sized materials (*in situ*) or after the synthesis of micro/nano sized materials they were subjected to drug incorporation. The drug loading efficiency of starch-based micro/nano DDS was determined as entrapment or encapsulation of the therapeutic agents in the starch carrier system. The entrapment/encapsulation deals with predicting the

concentration of the therapeutic agent in supernatant yielded after the fabrication of therapeutic loaded micro/nano DDS. While drug loading, the mean value obtained from the the concentration of therapeutic agent in supernatant was negligible from the total therapeutic concentration taken for the entrapment/encapsulation. The percentage of entrapment/encapsulation efficiency of starch-based micro/nano DDS was mostly calculated by using below formulae models.

$$\text{Encapsulation efficiency}(\%) = \frac{[\text{Therapeutic}]_{\text{Total}} - [\text{Therapeutic}]_{\text{Free}}}{[\text{Therapeutic}]_{\text{Total}}} \times 100$$

15.1

(or)

$$\text{Encapsulation efficiency}(\%) = \frac{[\text{Therapeutic}]_{\text{Free}}}{[\text{Therapeutic}]_{\text{Total}}} \times 100$$

15.2

where the $[\text{Therapeutic}]_{\text{total}}$ is the total concentration of therapeutic agent taken for the loading on starch-based micro/nano DDS and $[\text{Therapeutic}]_{\text{free}}$ is the unloaded concentration of the therapeutic agent available in the supernatant obtained from the fabrication of starch-based micro/nano DDS. Along with the therapeutic loaded efficiency the percentage of yield was calculated using the following formula

$$\text{Encapsulation efficiency}(\%) = \frac{\text{Total mass of starch DDS}}{[\text{Starch}]_{\text{total}} + [\text{Therapeutic}]_{\text{total}}} \times 100$$

15.3

Here the percentage yield for the starch-based micro/nano DDS was calculated by using the weight of the yielded starch-based DDS and the weight of starch ($[\text{Starch}]_{\text{total}}$), therapeutic agent ($[\text{Therapeutic}]_{\text{total}}$) taken for the fabrication of starch-based micro/nano DDS.^{24,48} Athira and Jyothi reported that curcumin loaded cassava starch nanoparticles exhibited a high loading efficiency (65%), enhanced cellular uptake, and nontoxicity toward L929 fibroblast cell lines and restored antioxidant efficiency. Chin *et al.*, reported fabrication of curcumin loaded starch nanoparticles by a nanoprecipitation method and water-in-oil microemulsion system which

showed 78% loading efficiency with improved water solubility.⁹⁹ El-Feky *et al.* reported better loading efficiency of insoluble drugs such as indomethacin (IND) and acyclovir (ACV) within starch nanoparticles.¹⁰⁰ Vitamin D3 was loaded in high amylose potato and corn starch nanocarrier by sonication methods. Results revealed a high vitamin D3 loading capacity of 94.8%, encapsulation efficiency, thermal stability, higher zeta potential, and a lower polydispersity index was observed in potato starch than corn starch (Hasanvand *et al.* 2015).¹⁰¹

The drug release percentage of starch-based micro/nano DDSs was evaluated mostly in a time and pH-dependent manner. Starch-based micro/nano DDS encapsulated with therapeutic agents have been reported for their drug release profile in certain *in vitro* and *in vivo* models. Briefly, the release percentage of the therapeutic agents at particular fixed time intervals was recorded in order to derive the drug releasing capacity of the starch micro/nano DDS. As we mentioned before, the swelling, surface erosions and solubility of the starch micro/nano DDS were the significant factors of drug release. Accordingly, most of the starch micro/nano DDSs were evaluated for their drug releasing profile under the physiological pH (7.4) fluid system, the adaptable pH in the human body. Here, physiological pH was adopted by using the *in vitro* saline system such as phosphate buffered saline (PBS) to evaluate the release percentage of the therapeutics.^{23,24,48,68} Otherwise, the *in vitro* drug release of the starch-based micro/nano DDS was carried out with different pH systems based on the targeted disorder to be treated with therapeutic loaded DDSs. Acevedo-Guevara *et al.* evaluated the curcumin release from starch NPs on simulated gastric fluid at pH 2 and intestinal fluid at pH 8.⁶³ Ding *et al.* fabricated 5-fluorouracil loaded starch NPs and observed complete release of 5-fluorouracil within 17 hours in simulated colonic fluid (pH 7).⁷² Along with simulated colonic fluid (pH 7.2), the simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8) were also taken for the evaluation of BSA (bovine serum albumin) release from the starch nanocarriers.⁷⁶ Chin *et al.* reported sustained release of curcumin from starch nanoparticles under physiological pH over a period of 10 days.²⁷ El-Naggar *et al.* fabricated cost-effective cross-linked starch nanoparticles as a DDS for diclofenac sodium (DS) with a nanoprecipitation technique using Tween 80 as a

surfactant which showed 95% drug loading efficiency and sustained release of drugs for 6 h at physiological pH.⁶⁰ Studies by Raveendran *et al.* revealed that doxorubicin loaded starch nanoparticles exhibited slow and sustained release of drug in PBS (pH 7.4) for 5–7 days indicating the starch polymer is an efficient cargo for chemotherapeutic drugs.¹⁰² Chong *et al.* reported the fabrication of piperine loaded starch NPs by a nanoprecipitation method which showed a high drug loading capacity and sustained release of piperine for 168 h under physiological pH.¹⁰³

The *in vivo* evaluations of the therapeutic release from micro/nano DDS were also carried out using the laboratory animal models. The concentration of the therapeutic agent in the biological fluids or tissues of animal models describes the *in vivo* drug releasing efficiency of starch-based micro/nano DDS. Jain *et al.* reported the *in vivo* release of insulin from starch NPs on male wistar rats induced with streptozotocin. *In vivo* release of insulin from starch nanoparticles in streptozotocin induced rats makes it applicable as a DDS for the treatment of diabetes.⁷⁸ Li *et al.* evaluated the *in vivo* release of morin on hyperuricemic rats administrated with morin conjugated hydroxyethyl starch–deoxycholic acid NPs.⁸⁰ The paclitaxel loaded pore starch NPs previously reported for its anticancer application was also evaluated for release of paclitaxel. At last, the oral administration of paclitaxel loaded pores with starch NPs in a Sprague Dawley rat model results in higher bioavailability of paclitaxel on rat plasma samples.⁶¹

The loading and release of therapeutic agents on starch micro/nano DDS implies its potential in therapeutic applications. Nanoencapsulation and microencapsulation of the therapeutics were also considered for immobilization of the therapeutics that we conferred before. Besides the starch-based micro/nano DDS has been reported with excellent loading of therapeutics due to its enhanced binding affinity with therapeutics. Moreover, the therapeutic release profile of the starch-based micro/nano DDS on various *in vitro* and *in vivo* models with respect to the physiochemical parameters ensures its futuristic prospect in therapeutic applications to overcome the various physiological disorders.

15.7 Conclusion

This chapter gives an overview of different types of plant sources for the massive extraction of native starch along with the amylose and amylopectin range on its composition. The percentage of amylose and amylopectin in a starch polymer system extracted from different tubers, cereals, and fruits were clearly categorized and tabulated. Physical, chemical, and biochemical factors involved in starch modification in order to alter its texture, morphology, size, and binding structure were also discussed. The above-mentioned factors on starch modification played a major role in the fabrication of starch-based micro/nano DDS. With reference to the above, it is confirmed that the combination of the modifying factors and processing techniques has also accounted for the fabrication of starch-based micro/nano DDS through various techniques such as microemulsion, graft copolymerization, ultrasonication, nanoprecipitation, high pressure homogenization, and hydrolysis. This chapter also tabulated the therapeutic benefits of starch-based micro/nano DDS on antimicrobial, anticancer, antioxidant, and neuroprotective applications. Nevertheless, the chapter detailed the therapeutic potential of starch-based micro/nano DDS with respect to the entrapment/encapsulation of various natural and synthetic therapeutic agents like curcumin, insulin, resveratrol, quercetin, gallic acid sources, *etc.* In addition to the therapeutic applications, this chapter explained the kinetics of therapeutic release from the micro/nano DDS on various simulated fluids, buffer systems, and animal administrations using *in vitro* and *in vivo* model systems. The collective observation based on the previous reports explained about cell viability, cell mediated toxicity, cellular uptake, biofluid permeability, enzyme inhibition, microbial suppression, biodegradability, therapeutic loading, and release aspects of the starch-based micro/nano DDS; acknowledging that the starch-based micro/nano DDSs are reliable for therapeutic application in the present inventions and futuristic prospects. Hence, the present chapter will be useful for the understanding of source, routes, and application of starch-based micro/nano DDS.

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Chapter 16

Tamarind Seed Polysaccharide in Novel Drug Delivery and Biomedical Applications

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16.1 Introduction

Tamarind seed polysaccharide and its functionalized derivatives have been extensively used throughout the globe prompting many researchers to improvise upon the existing or published research whether it may be in terms of its novel applications or tweaking its structure to make new derivatives or combining it with hitherto never used natural or synthetic polymers. We have tried to put forth comprehensive applications of tamarind seed polysaccharide/gum/xyloglucan or galactoxyloglucan in terms of the erstwhile novel drug delivery systems and recent applications in esoteric and futuristic novel drug delivery systems (NDDS) and biomedical applications published in the literature world-wide. At the same time the chapter puts forth the safety aspect, some trade names which are more popular, and has also touched upon its basic and relevant physicochemical properties. Readers affiliated to Pharma and health-related fields will consider it as a go-to chapter for tamarind seed polysaccharide.

16.1.1 Synonyms

Tamarind gum, tamarind seed gum, tamarind kernel gum, tamarind seed xyloglucan, and tamarind galactoxyloglucan (storage xyloglucan)

16.1.2 Botanical Source and Chemical Structure

Tamarind seed polysaccharide (TSP) is a GRAS listed high molecular weight (400–6000 kDa) natural branched polymer.^{1–3} It is produced as a major product from the kernels by dehulling and pulverizing the seeds of *Tamarindus indica* L. which are indigenous to tropical Africa and widespread in Asia and Central America, India and Thailand being the predominant producers.⁴ *Tamarindus* is a monotypic genus containing the sole species *T. indicus* and belongs to the sub-family *Caesalpinioideae* of the family *Fabaceae* (*Leguminosae*).^{3,5} Tamarind xyloglucan an important component of primary cell walls functions as a storage polysaccharide.^{6,7}

Besides polysaccharide, which accounts for about 65% of the weight of the seed, water, protein, unsaturated fatty acids, and tannins are also important constituents of tamarind seeds. It's

polysaccharide consists of a cellulose-like backbone composed of a linear chain of α -glucose units linked by $\beta(1-4)$ glycosidic bonds.^{3,8} Single α -xylose units are attached to about 75% of these α -glucose units *via* $\alpha(1-6)$ bonds. The additional molecular side chains differentiate tamarind seed gum from other xyloglucan sources, as here the xylose units are attached to a galactose unit by $\beta(1-2)$ linkages (Figure 16.1).^{1,3}

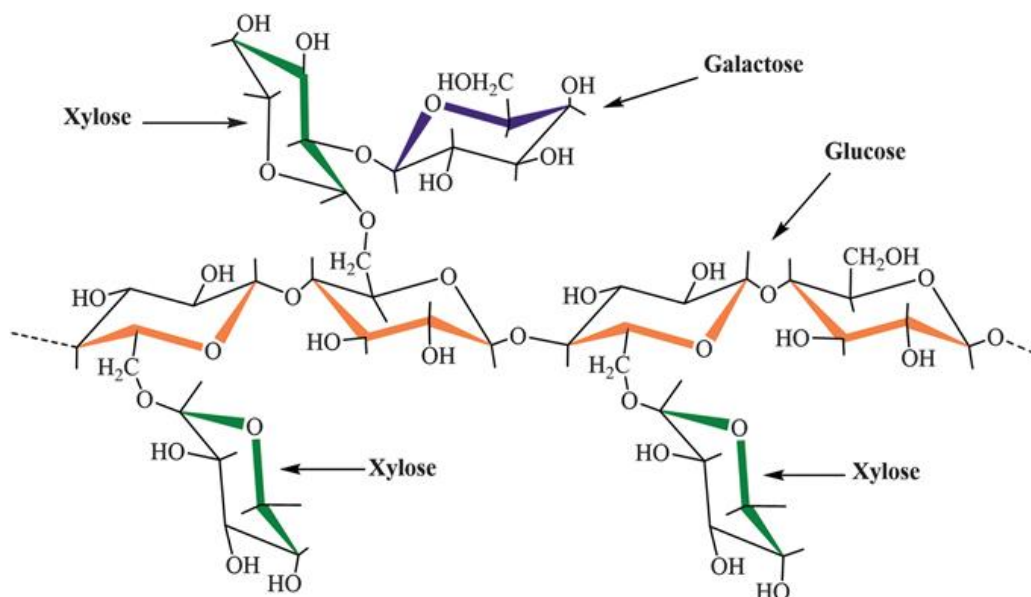


Figure 16.1 Chemical structure of tamarind seed polysaccharide.

Though a topic of discussion, the molar ratio of glucosyl : xylosyl : galactosyl in the polysaccharide has been reported to be varied; 3 : 2 : 1, 4 : 3 : 1, 4 : 2 : 1, 2.9 : 1.8 : 1, 2.8 : 2.25 : 1.0 (43–45% glucose, 35–38% xylose, 15–17% galactose) and even 2.61 : 1.43 : 1 or 7.8 : 4 : 2 : 1.2 by some researchers, the fourth component; l-arabinose (2–3%),^{1,3,7,9–14} thus indicating that it is a galactoxyloglucan.³ Recently its fine characterization by GC-MS analysis, 2D NMR, high performance size exclusion chromatography (HPSEC) *etc.* revealed that its molar ratio is 3.1 : 1.7 : 1.0 with a molecular weight of 524.0 kDa.¹⁵

Differences in the composition of TSP have been found in various literature reports^{3,5,16–18} depending upon the origin, purification method, and purity of gum (Table 16.1).

Table 16.1 Composition of TSP.

Compound present	Percentage
Non-fiber carbohydrate	65.1–72.2
Protein	15.4–22.7 (38.7% is insoluble protein and 61.3% is soluble)
Oil/lipids	3.5–7.5
Crude fiber	0.7–8.2
Total ash (on a dry basis)	0.7–3.3
Moisture content (%)	10–11%
LoD (105°, 5 h)	NMT 14%

16.1.3 Selective Properties of TSP

TSP is a free-flowing, tasteless powder that is white or light brown in color and may be odorless or have a slight greasy odor. It is insoluble but dispersible in cold water and insoluble in most organic solvents including ethanol, methanol, acetone, and ether.^{3,16,19,20} However, a cold, aqueous solution of the polysaccharide when heated to 85 °C results in the formation of a uniform solution.^{21,22} X-Ray diffractograms have indicated the amorphous nature of TSP.⁸ Table 16.2 sums up the physicochemical properties of TSP as reported in various literature reports.^{1,12,13,16,19,22,23}

Table 16.2 Physicochemical properties of tamarind seed gum/polysaccharide.

Property	Value
Molecular weight (kDa)	400–6000
Viscosity average molecular weight (g mol^{-1})	980 000
Average intrinsic viscosity (ml g^{-1})	600 ± 0.05
Linear viscoelasticity	0.63–6.37 Pa of oscillatory shear stress
Viscosity (mPa s)	400–800 (150 cSt of 1% w/v at 30 °C)
True density (g cc^{-1})	1.015–1.9
Bulk density (g cc^{-1})	0.24–0.65
Tapped density (g cc^{-1})	0.363–0.781
Compressibility index (%)	15.33–16.64
Angle of repose	13–30°
Hausner's ratio	0/14–1.23
Particle size (μm)	60–90
pH (1% w/v)	6–6.81
Swelling index (in water)	12–17
Surface tension (dynes per cm)	61.3–83.26
Water retention (%)	20 ± 1.34
Melting point (°C)	240–260
Refractive index	1.334

The average molecular weight of TSP has been reported by various research groups from 470–5870 kDa based on the method of purification and detection.^{2,24,25} The viscosity of tamarind seed gum depends on its concentration and the corresponding temperature. At low concentrations, its viscosity is only temperature dependent, exhibiting distinct shear thinning behavior at higher concentrations ($>0.5\% \text{w/v}$).^{2,5,12,26,27} Recently Shao H. *et al.* have studied its rheological properties and have found that apparent viscosities and viscoelastic parameters of TSP solutions decreased significantly in an alkaline solution of $\text{pH} > 10$, being stable at neutral pH, slightly influenced by an acidic environment, high temperature, and the presence of salt ions and sucrose.²⁷ The maximum gel strength for a solution with 1% of polysaccharide and 50% sugar has been reported to be at pH 2.8.^{28,29}

In isolation it does not form a gel in aqueous solution even at high concentrations, forming only a viscous solution, but on addition of other polysaccharides, such as gellan and xanthan gum, or cross-linkers such as congo red, or on addition of green tea-extract polyphenols such as epigallocatechin, or on addition of large amounts of sugars (40–70%) like sucrose, glucose, or starch and also alcohol (monohydric/polyhydric), aqueous solutions of xyloglucan form a thermoreversible gel at certain temperatures or under particular conditions.^{2,30–35}

Recently Minghui and his co-workers studied the effect of temperature (30, 55, and 80 °C) on the structural and physicochemical properties of TSP after its extraction from its seeds. It was concluded by them that all the extracted TSP contained starch, the one extracted at 80 °C qualifying as the best for its applications in drug delivery.³⁶

16.1.4 Safety

Tamarind seed polysaccharide was considered by the Joint FAO/WHO Expert Committee for Food Additives at its June 2017 meeting. The committee observed absence of toxicity in long-term rodent studies and lack of concern regarding genotoxicity, reproductive toxicity, and developmental toxicity. The committee concluded estimated dietary exposure of 75 mg kg⁻¹ body weight per day based on proposed uses.³⁷ TSP has been commercially available as a permitted food additive in Japan since 1964.^{24,38} TSP is Generally Recognized as Safe. GRAS Notice Inventory No. 503 addresses the use of TSP as a thickener, stabilizer, emulsifier, and gelling agent in several food categories at concentrations ranging from 0.2–1.5% of product composition. Tamarind seed gum is on the EPA's 2016 Chemical Data Reporting full exempt list.^{24,28}

16.1.5 Commercial Products of TSP

There are very few companies manufacturing food, cosmetic, and pharmaceutical grade tamarind seed polysaccharide worldwide.^{38–41} The grade Glyloid from DSP Gokyo, Japan is the leading product covered by GRAS Notice No. 503 for TSP. Details of the products are given in Table 16.3.

Table 16.3 Details of companies manufacturing TSP and its different grades.

S. no	Name of the product	Category	Important properties	Manufacturer
1	GLYLOID DSP	Gokyo Food and Chemical Co. Ltd, Japan		
	GLYLOID 2A	Hot water-soluble	Requires heating at 75 °C for at least 15 min for solubilization	DSP Gokyo Food and Chemical Co. Ltd, Japan
	GLYLOID 3S	Cold water-soluble	Forms a viscous solution in cold water without heating	DSP Gokyo Food and Chemical Co. Ltd, Japan
	Glyloid 6C	Cold water-soluble	Highly transparent	DSP Gokyo Food and Chemical Co. Ltd, Japan
	GLYATE	Acid-hydrolyzed	Much lower viscosity, ranging from 1 to 10 mPa s, compared to over 400 mPa s for non-hydrolyzed TSP	DSP Gokyo Food and Chemical Co. Ltd, Japan
2	Xilogel	(Xilogel [®] – Indena)	For cosmetic use	Indena Spa, Milan Italy
3	Unitamuron:H-22	Pentylene Glycol and TSP	For cosmetic use	Induchem, USA and France

16.2 Pharmaceutical Applications of TSP and Chemically Modified TSP

An erstwhile under-utilized by-product of the tamarind pulp industry until the mid to late 1900s, tamarind seed polysaccharide in the last 4–5 decades gained popularity due to its application in the food and specifically the pharmaceutical industry.⁴² This is mainly due to its excellent properties like biocompatibility, biodegradability, good stability, non-carcinogenicity, broad pH tolerance, excellent drug holding capacity, high swelling capacity, easy availability and reasonable cost, establishing its dominance in various conventional dosage forms, NDDS and currently for various biomedical applications.⁴³

Though TSP has wide applications in various drug delivery systems it suffers from drawbacks such as unpleasant odor, dull color, poor aqueous solubility, and ability to degrade in an aqueous environment. To overcome these shortcomings tamarind gum has been chemically modified using various functional groups such as carboxymethyl (CMTSP), acetyl, cyano-ethyl, sulfate, alkyl, thiol, polymer grafting, degalactosylation, and even cross linking (Figure 16.2).^{44–49} The most popular derivative, CMTSP has carboxymethyl groups replacing hydroxyl groups which disrupts the organization of the structure, confers an anionic nature to the biopolymer, and enhances the networks' hydration ability resulting in higher solubility in aqueous media.^{44,50}

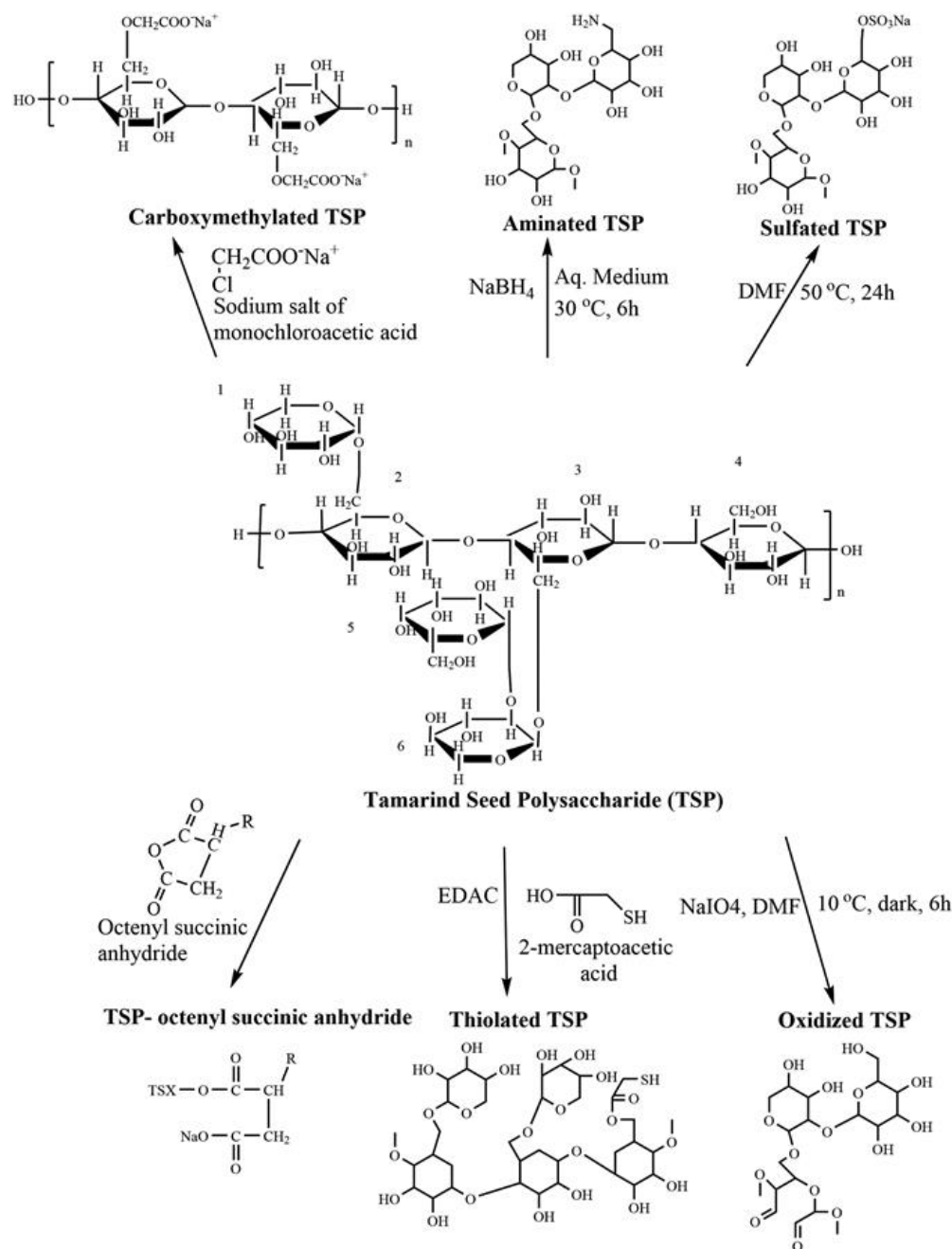


Figure 16.2 Modification of TSP, NaIO_4 : sodium periodate, TSP: tamarind seed polysaccharide, DMF: dimethyl formamide, NaBH_4 : sodium borohydride, EDAC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

These functionally modified TSPs thus hold great potential as pharmaceutical excipients in a variety of novel drug delivery systems because of their improved stability and enhanced mechanical behavior, as well as competence in prolonging drug release. Some of these derivatives have exhibited higher viscosities, better solution stability (shelf-life), resistance to biodegradation, and clarity as compared to TSP.^{49–51}

16.3 Applications of TSP in Conventional Drug Delivery Systems

TSP has been investigated as a multifunctional excipient indicating the versatility of a natural polymer not only in the conventional dosage forms but also in NDDS. It has been utilized as a suspending agent (paracetamol), a binding agent (ibuprofen tablets), is a better emulsifying agent as compared to gum acacia, and as a binder and release retardant together for terbutaline sulfate.⁵²⁻⁵⁵

At the same time the functionalized TSPs have also found applications in the simplest of the DDS, as a tablet binder, as a carrier in solid dispersions, for taste masking, and as a disintegrating agent. Huanbutta *et al.* have used CMTSP as a dry binder in diclofenactablets.⁵⁶ Thai cordial, a traditional Thai herbal medicine was used as a model drug to study the fast disintegrant properties of CMTSPs.⁵⁷ They have also fabricated taste masked oral disintegrating tablets (<2 min) by freeze drying using CMTSP as a binding agent along with mannitol.⁵⁸

In one of the studies, TSP and CMTSP were compared to see their effect as a wall material to microencapsulate citronella oil by spray-drying. CMTSP microcapsules had a smaller emulsion droplet size, smoother surface, spherical shape, and higher entrapment efficiency (EE) than TSP microcapsules, the maximum EE (87%) being observed at a 1.14 : 1 gum to oil ratio.⁵⁹

Solid mixtures of Celecoxib and TSP were prepared in different ratios; 1 : 1, 1 : 4, and 1 : 9 w/w, using co-grinding, physical mixing, kneading, and solvent deposition methods to determine the effect of the method of preparation on its dissolution rate. The dissolution rate of drug increased with increasing TSP concentration but the optimum ratio was concluded to be 1 : 4 (Celecoxib : TSP). It was found that the order of mixtures based on dissolution efficiency was solvent deposition > co-grinding > kneading > physical mixing > pure drug, co-grinding being more suitable from a practical point of view and commercialization aspect.⁶⁰ Recently Famotidine solubility was enhanced using TSP and hyaluronic acid (1 : 1) in the form of their solid dispersions.⁶¹

16.4 Applications in Novel Drug Delivery Systems and Biomedical Fields

TSP and modified TSP have been used alone and in combination with various natural and synthetic polymers for various NDDS; mucoadhesive polymer in various dosage forms, as a release retardant excipient, as a film former and in *in situ* gels for nasal, ophthalmic, periodontal drug delivery, *etc.* It is finding potential use as nanoparticles, nanocomposites, nanoaggregates, hydrogels, scaffolds *etc.* in NDDS and the biomedical field, specifically in tissue engineering and theranostics, in its natural and modified form, as well as, after grafting it with other polymers, so as to further overcome its disadvantages like slower gelation time, reduced viscosity, biodegradability, and stability on storage.

16.4.1 Novel Drug Delivery Systems

16.4.1.1 Applications as a Mucoadhesive Polymer (Tablets/Films/Patches)

TSP has various features which makes it an attractive polymer candidate for applications as a viscosity enhancer and for mucoadhesive or bioadhesive activities. Conformation of xyloglucan imparts TSP a 'mucin-like' molecular structure making it ideal for mucoadhesion, its strength depending on the concentration used coupled with its high swelling property.^{62,63} These features have been utilized by formulators in making NDDS for targeting various routes. In some cases the mucoadhesive property has been combined with an *in situ* gelling property to have a better DDS. It becomes an ideal candidate for bioadhesion in buccal films/patches and tablets, in topical gels,

transdermal films (alfuzocin HCl), for *in situ* nasal gels (Mirtazapine) and even for colon targeted drug delivery without the use of pH-dependent polymers.^{64–67}

16.4.1.1.1 Oral

In one of the reported studies the authors put TSP's ability to form a film to good use by preparing mucoadhesive oral films for aphthous stomatitis, a side effect of cancer chemotherapy. TSP and HPMC as a plasticizer were combined in various ratios to prepare these films loaded with loperamide, a BCS class-IV molecule. They achieved good adhesion, strength, and desired release from these films.⁶⁸

16.4.1.1.2 Oesophageal

With the aim of providing a physical barrier to block the diffusion of acid, pepsin, and bile acids from the stomach across the esophagus and to reduce the erosive effect of these physiological fluids in Gastro-esophageal reflux disease this study was recently undertaken. Chondroitin sulfate (ChS) and TSP along with glycerine were combined, utilizing the protective effect of ChS in gastric ulcer and mucoadhesive properties of TSP to develop AL2106 a Class III medical device distributed by Alfasigma SpA (Bologna, Italy). It was compared with Esoxx One, a similar medical device already on the market in many countries. AL2106 was found to have a better performance in terms of the duration of the barrier effect compared to Esoxx One. The device is liquid when swallowed, but rapidly its viscosity increases, adhering immediately to the esophageal mucosa. Both were evaluated for their rheological behavior, buffering capacity against acidity, mucoadhesive properties, and film-forming ability with the consequent protective effect on esophageal mucosa. It scored over 'Esoxx one' in all these aspects, the barrier effect lasting for 5 h showing a long lasting protective effect.⁶⁹

16.4.1.1.3 Buccal

TSP has been utilized as a potential mucoadhesive polymer for buccal delivery of drugs like metoprolol succinate, rizatriptan *etc.*⁷⁰ A highly soluble anti-migraine drug; rizatriptan benzoate along with Carbopol 934 was formulated as a buccal patch which was able to deliver the drug for the required time and rate. 93.45% of drug was able to diffuse through this bilayer film containing 4% (w/v) TSP and 0.5% (w/v) Carbopol using porcine buccal mucosa.⁷¹ Buccal patches containing benzydamine HCl and lidocaine were made using TSP as a mucoadhesive agent from which sustained release was achieved for 8 h with zero order kinetics. Salification of benzydamine HCl with acidic polymers; polyacrylic acid or pectin was carried out to control the release characteristics.⁷² It has also been cross-linked with epichlorohydrin for the development of buccal metronidazole patches.⁷³

Bioadhesive gels containing hydrophobic drug; itraconazole loaded microemulsions using various gums including TSP for its delivery were investigated to treat fungal infections with an improved therapeutic outcome. They were evaluated for *ex vivo* bioadhesion, viscosity, drug release and its kinetics, and antifungal activity. A ternary phase diagram was constructed for microemulsion consisting of isopropyl myristate and oleic acid as the oil phase, tween 80 as the surfactant, and isopropyl alcohol as the cosurfactant. Amongst the studied gels, TSP (2% w/v) containing gels exhibited faster and complete drug release at the end of 24 h and were found to be stable for three months.⁷⁴ Kaur *et al.* prepared cationic (amine) and anionic (carboxymethyl, sulfate) derivatives of TSP and evaluated and compared them for their bioadhesive potential. TSP (1% w/v) buccal films were prepared by a solvent casting method. These functionalized polysaccharides were found to be more hydrophilic and exhibited greater swelling than TSP, and displayed higher force of detachment

as compared to TSP; aminated TSP required the highest force of detachment from porcine cheek mucosa.⁷⁵

16.4.1.1.4 Ophthalmic

It has been suggested that TSP may be useful in ophthalmic delivery systems for topical administration of antibiotics⁷⁶ and also as an adjuvant.⁷⁷ Tear fluid substitute containing TSP for dry eye syndrome is one of the products which is on the market (HydraMed: Farmigee S.p.A., Italy)⁷⁸ due to its activity in preventing alterations of the corneal surface. This is possible because of its structural similarity to mucin as stated earlier.⁶³ It is devoid of ocular toxicity, improves corneal-wound healing, and diminishes *in vitro* toxicity of human conjunctival cells.⁷⁹ Timolol was found to be suitable for ocular administration in cases of increased intraocular pressure (IOP) showing prolonged duration of action when 1–2% TSP was used.⁸⁰

Burgalassi *et al.* filed a patent for preparing viscofied solution of TSP which demonstrated pseudoplastic behavior, could function as artificial tears (0.75–1.5% w/v TSP) with a mucoadhesive nature, and could sustain the release (1–4% w/v TSP) of added drug, depending on the concentration of TSP in ophthalmic delivery.⁸¹ Burgalassi *et al.* have verified the performance of TSP as an excipient for ophthalmic preparations containing timolol in comparison to two commercial formulations (standard aqueous eye drops and an *in situ* gelling formulation), all containing same amount of timolol. Ocular pharmacokinetic studies and plasma level measurements were carried out on pigmented rabbits; the pressure-lowering effect in normotensive pigmented rabbits was also evaluated. TSP formulation showed a significantly more prolonged IOP depressing activity in the tested rabbit model compared to the reference formulations. It was further confirmed that mucoadhesive delivery systems can substantially reduce the systemic absorption of timolol, compared to traditional, non-viscous eye drops.⁸²

Ghelardi *et al.* inferred from their study that TSP can enhance trans-corneal disposition (with proven intraocular safety) of both hydrophilic and hydrophobic antimicrobial drugs, such as gentamicin and ofloxacin, when administered by an ophthalmic route to healthy rabbits by an ocular drop regime as there was increased drug absorption and prolongation of the drug elimination phase obtained with TSP-based viscous formulations.⁷⁶ Furthermore, E. Ghelardi and co-workers assessed the effect of TSP on the ocular delivery of 0.3% rifloxacin for treatment of *P. aeruginosa* and *S. aureus* induced keratitis in rabbits and compared it against standard ofloxacin treatment. TSP significantly increased intra-aqueous penetration of rifloxacin in both infected and uninfected eyes, bringing about a reduction in bacterial count in cornea at a higher rate than that obtained by only rifloxacin. The results also indicated that TSP extends drug's precorneal residence time and enhances drug accumulation in cornea.⁸³

Stable, non-irritating ocular films of Timolol maleate were developed using TSP as a film former as well as a release retardant material. The thickness of the ocular films ranged from 0.17 to 0.25 mm. Drug release from them was controlled over a period of 8 h and they were able to reduce the IOP for 24 h in a more efficient manner than the eye drops.⁸⁴ Rolando *et al.* have shown the potential benefits of TSP (1%) over hyaluronic acid (0.2%) in treating dry eye syndrome with respect to trouble in blinking, ocular burning, and foreign body sensation.⁸⁵ Using the synergy of both HA and TSP researchers have worked on their combined formulations for ophthalmic use. They found that a 3 : 2 ratio of TSP : HA demonstrated greater mucoadhesivity in comparison to both individual components and other formulation ratios.⁸⁶ Its ability to generate non-covalent interactions with hyaluronic acid was demonstrated and exploited to develop artificial tears for dry eye syndrome with

enhanced mucoadhesive properties using diclofenac sodium. TSP/HA in equal ratios produced a stable aggregate with better mucoadhesive nature due to their synergistic interactions.⁸⁷

Based on the medical benefits of combining HA and TSP for treating dry eye disease as reported by Barabino *et al.*, and then by various authors as above, Chun *et al.* have explored its use as a supplement for lachrymal secretion/ocular mucin, which diminishes with aging in dry eye.^{88,89} To prolong the retention time of HA, it was combined with TSP not only to understand the hydrodynamic characteristics of HA and TSP but also to explore the compatibility of these polymers, including the possibility of harmful aggregation effects. Hardly any evidence of aggregation based on various hydrodynamic characteristics was found by the authors encouraging them to develop eye-drop formulation technology comprising these substances.^{88,89}

Dilbaghi *et al.* have formulated and characterized CMTSP-based nanoparticles for ophthalmic delivery of tropicamide. Tropicamide loaded CMTSP was cross linked by CaCl_2 using an ionotropic gelation method and the optimized nanosuspension after freeze drying was evaluated for *ex vivo* corneal permeation besides other physicochemical properties. Concentration of CMTSP and CaCl_2 had significant synergistic effects on particle size and encapsulation efficiency (EnE).⁹⁰

16.4.1.1.5 Nasal/olfactory

The fact that drugs through a nasal route can directly cross the blood brain barrier to the target brain have been explored. However, retention in the olfactory region requires a mucoadhesive excipient and if it is from a natural origin it scores over synthetic polymers. With this view TSP has been utilized for a nasal/olfactory drug delivery system so as to have direct nose to brain delivery.

It has been tried for diazepam, and found to be better with respect to mucoadhesive strength, viscosity, gelling properties, and *ex vivo* diffusion studies in comparison to synthetic polymers like HPMC or carbopol 934.⁹¹ Recently Rizwan and co-workers have developed fluorescently-labeled dextran loaded TSP microparticles for nasal drug delivery. Based on the hypothesis that particles of 10 μ size show maximum deposition and retention in the olfactory region TSP was spray dried to achieve the targeted sized microparticles (10 μ), wherein they concluded based on *ex vivo* release, permeability studies, and deposition studies that 10 μ sized particles had maximum retention on olfactory mucosa and aids in transport of drugs across the brain.⁹²

16.4.1.1.6 Pulmonary

Novel polymeric microspheres of Montelukast sodium were developed using TSP to prolong the retention of anti-asthmatic agents in lungs using a spray drying technique. TSP microencapsulated Montelukast had better mucoadhesive properties as a DPI.⁹³ Furthermore, these authors have prepared Rifabutin microspheres in a similar manner for the treatment of tuberculosis with 2% TSP and 1% lactose monohydrate which were found to be most favorable. Their *in vitro* performance, morphology, and particle size distribution demonstrated the suitability of these microspheres as DPI for pulmonary delivery.⁹⁴

16.4.1.1.7 Topical

Recently the use of organic and inorganic fillers has caught the eyes of various research groups as they have enhanced thermal and mechanical properties, bentonite being one of them. In one of the studies PVA/TSP along with bentonite composite films were developed using Ciprofloxacin as a model drug. They were able to obtain films with excellent optical and swelling properties, so as to give sustained release of Ciprofloxacin. Release was governed by the interaction between TSP and

bentonite as this could have increased the water absorption capacity of the films and hence the ability of drug molecules to diffuse. The films demonstrated a good antimicrobial effect against both *E. coli* and *B. subtilis*.⁹⁵

16.4.1.1.8 Vaginal

TSP bioadhesive films were prepared employing a solvent casting method containing propylene glycol as a plasticizer, which were tested as drug carrier systems for vaginal delivery of nystatin.⁹⁶

16.4.1.2 In Situ Gelling Systems

In situ gelling systems for oral, nasal, rectal, and ophthalmic delivery have been attempted by various research groups. An *in situ* gelling TSP and pectin mixture for oral administration of paracetamol was attempted by Attwood *et al.* The desired release rate and higher bioavailability were achieved using 1.5% (w/w) xyloglucan and 0.75% (w/w) pectin.⁹⁷

Pilocarpine thermoresponsive *in situ* gelling solutions based on sodium alginate along with tamarind gum or chitosan (CH) were formulated. Based on *in vitro* as well as *in vivo* studies it was reported that TSP based gums had the best of the requisite properties for ocular delivery and it was able to deliver 80% drug in 12 h.⁹⁸ Incorporation of the same drug 'Pilocarpine' was done in another study where the authors have used TSP along with Poloxamer 407 as an *in situ* ophthalmic drug carrier. They have recommended a combination of 0.5–1.5 wt% TSP and 18 wt% poloxamer solution as they brought about significant improvement in gel elasticity under physiological conditions, enhanced the viscosity of gel, and also stimulated the gelation procedure. TSP was used to improve the thermogelation property and drug holding capacity of the formulations.⁹⁹

Sustained release properties of pilocarpine loaded TSP *in situ* gels were compared with the release characteristics of a Pluronic F127 gel formed *in situ* under identical conditions in the rabbit eye. Changes in pupil diameter were used to monitor the pharmacologic response. Rheological properties before and after instillation of *in situ* gels were monitored carefully to understand the retention in the precorneal cavity. The degree of enhancement of the miotic response of pilocarpine from 1.5% w/w tamarind gel matched with that obtained from a 25% w/w Pluronic F127 gel both showing sustained release.¹⁰⁰

Sodium alginate was combined with TSP to assess the delivery of Zolmitriptan and ketorolac tromethamine together using the concept of thermoresponsive *in situ* nasal gel. TSP (~ 1%) along with sodium alginate and Pluronic F 127 (20%w/v) gave increased bioavailability based on *ex vivo*, *in vivo* studies, while histopathological studies assured the safety of its use by a nasal route.¹⁰¹ Mali *et al.* have prepared and evaluated *in situ* nasal gel for Granisetron HCl using CMTSP. They concluded that CMTSP is better in terms of ease of administration, accuracy of dosing, prolonged nasal residence time, and improved nasal bioavailability.¹⁰²

Mahajan *et al.* reported on the synthesis and characterization of thiolated TSP for its *in situ* gelling and mucoadhesive application for nasal administration of ondansetron. Based on *ex vivo* studies using nasal mucosa they concluded that thiolated TSP had better mucoadhesion as well as permeation enhancing properties.¹⁰³ The antiepileptic drug Rufinamide suffers from very poor bioavailability necessitating frequent administration for pediatric patients. *In situ* nasal gel using 2% w/v TSP as a gelling and mucoadhesive polymer was formulated for achieving nose to brain delivery, thus increasing bioavailability and reducing dosing frequency.¹⁰⁴

Besides all these routes, researchers have found application of the mucoadhesivity of TSP in the form of *in situ* gels for dental diseases, specifically to target periodontal pockets for drugs like

lidocaine.¹⁰⁵ To treat periodontitis 24 hour sustained release metronidazole loaded *in situ* gels comprising of various ratios of TSP and Pluronic F127 were prepared and characterized by SEM, DSC, rheological analysis, and *in vitro* release studies to finally conclude that 0.1% TSP and 18% Lutrol containing gel had the requisite characteristics.¹⁰⁶

Suisha *et al.* have examined the potential of *in situ* gelling behavior of TSP (partially degraded by β -galactosidase)¹⁰⁷ (1–2% w/w) for rectal administration of indomethacin as well as Diltiazem HCl and they concluded that TSP gel has a longer residence time and a more sustained release as compared to commercially available suppositories. Plasma levels of indomethacin after rectal administration of TSP gels to rabbits indicated a broader absorption peak, the residence time being longer than the control product.¹⁰⁸ The same system and concept for both these drugs were also attempted successfully by an oral route.¹⁰⁹

Gallic acid (0.6% w/v) and TSP (0.8% w/v) combination along with NaCl was used as a thermoresponsive *in situ* gel for doxycycline hydrochloride for wound healing. The prepared formulation exhibited excellent stability and compatibility. This formulation in the treated group of rats showed faster epithelialization and higher rates of wound contraction.¹¹⁰

16.4.1.3 Oral Controlled Release Systems

OCRS have been developed for both water soluble and water insoluble drugs by tailoring the concentration of TSP and/or the diluents. Researchers have developed tablets and multiparticulate systems for sustaining the drug release and this has been attempted by both using TSP as well as modified TSP.¹¹¹

16.4.1.3.1 Tablets

TSP has been used alone for drugs like Diclofenac sodium, Verapamil, Glimepiride, and Naproxen while some workers have combined it with natural or synthetic polymers to achieve the desired release profile most of them being in the matrix system for *e.g.* Lamivudine, caffeine, paracetamol, theophylline, and indomethacin.^{112–116} They have studied the mechanism of drug release from the TSP matrix and the effect of varying concentrations of diluents and lubricants on their release profile. In several studies it has been concluded that as the concentration of TSP increased the sustaining effect also improved.^{117,118}

Researchers have converted their finding that CMTSP is better than TSP for application in an oral controlled release formulation of Diclofenac sodium tablets to achieve better physicochemical and functional properties over crude gums.¹¹⁹ Investigations have also been done for the use of TSP in sustaining the Metformin HCl layer (12 h) in a bilayer tablet where the second layer consisted of Sitagliptin phosphate for immediate release.¹²⁰

TSP with different concentrations has been used as a drug carrier for the delivery of paclitaxel for studying the *in vitro* drug release by changing the time, pH, and drug concentrations. Based on the diffusion as well as the kinetics, the mechanism of drug release from the composite matrix was reported by Sahoo *et al.*¹²¹ A matrix sustained release (24 h) tablet for Tramadol by direct compression has been developed by Kumar *et al.* which contained 30% TSP.¹²² A slightly different approach has been used by Pandit *et al.*, where the immediate release of the Tramadol HCl layer was segregated from the TSP–drug matrix layer by a drug free TSP layer. This ensured dual release of the drug, immediate release within half an hour, and then sustained release for 10 h.¹²³

It has also been cross-linked with epichlorohydrin for sustaining the release of salicylic acid from its tablet for 24 h. Drug release was found to depend on the efficiency of the cross-linking, where

higher drug permeation was observed with lower levels of cross-linking.¹²⁴ In order to prove the superiority of TSP, it was compared with two other natural polymers viz.; okra gum and CH in controlling the release of model drug; propranolol from their matrix tablet for its colonic delivery. TSP was found to be the best amongst all, its release following the Korsmeyer–Peppas model.¹²⁵ Singh *et al.* have developed and evaluated a chronotherapeutic drug delivery system by compression coating consisting of 67.5% TSP and Nifedipine as the core which resulted in 97% drug release in 12 h.¹²⁶

Sustained release formulations using CMTSP for Glipizide SR tablets (20 h) using direct compression have been attempted by Manchanda *et al.*,¹²⁷ and TSP with HPMC K 100M for tramadol HCl sustained release tablets (10 h).¹²⁸ Methylene blue was used as a model drug where TSP and CMTSP were compared. Kinetics revealed that CMTSP was better as it showed pH independent release for 70% of the time period. It followed a near zero order release pattern in the initial hours followed by non-Fickian diffusion for the remaining 60% of the drug release period.¹²⁹

16.4.1.3.2 Microparticulate Systems

CMTSP has also been used as a controlled release agent in the form of pellets or spheroids. Diclofenac sodium spheroids (~ 0.7 mm) containing MCC and TSP (10%) made by hot melt extrusion were studied. *In vitro* release studies exhibited zero-order release kinetics as confirmed by Higuchi and Peppas models. From the bioavailability studies it was inferred that sustained release of the drug was achieved for 8 h with improved absorption as compared to the marketed product.¹³⁰

CMTSP was loaded with Lansaprazole and converted to pellets via an extrusion–spheronization technique. Formulation also consisted of MCC and NaCMC whose concentrations were optimized. The effect of speed and duration of spheronization was observed and the authors were able to manufacture a stable product with release properties similar to the marketed product.¹³¹ In a similar way sustained release (12 h) pellets for lornoxicam have been developed and characterized.¹³²

Bhalekar and co-workers have synthesized a couple of derivatives of TSP for a variety of drugs. Their objective was to prepare and optimize sustained release mucoadhesive multiparticulate system of glipizide using TSP and its thiolated derivative using sodium trimetaphosphate as a crosslinking agent. Microspheres consisting of thiolated xyloglucan showed better EE (90%), *in vitro* release rate and mucoadhesion, as well as, showed significant reduction in blood glucose levels thus proving to be a more suitable polymer for multiparticulate drug delivery of glipizide.¹³³ This was an improvement over their earlier reported work where they have used only TSP for sustaining the effect of glipizide using sodium alginate as a gel-forming polymer which was made by the orifice-ionic gelation method.¹³⁴

Pandit and co-workers have explored a couple of applications of CMTSP for the formulation of pellets prepared by an extrusion spheronization technique for colon targeted drug delivery, one being ibuprofen¹³⁵ for colorectal cancer and the other curcumin¹³⁶ for inflammatory bowel disease. Ibuprofen was loaded as such while curcumin was converted into its solid dispersion using Soluplus. Ibuprofen was released over a period of 9 h and the pellets underwent diffusion first followed by erosion while the release behavior of curcumin pellets showed a swelling behavior followed by erosion. There was a two-fold increase in bioavailability from these pellets as compared to simple pellets.^{135,136}

Jyosna *et al.* have developed TSG and sodium alginate composite microspheres for sustained release (12 h) of Dalframipidine, a drug for multiple sclerosis. Sodium alginate with its burst release problems and TSP with its highly hydrophilic nature were combined to overcome their respective

functional drawbacks. Various parameters like polymer ratio, agitation speed, and concentration of CaCl_2 were optimized to obtain the desired formulation.¹³⁷

16.4.1.4 *Gastroretentive DDS*

TSP has also been used for manufacturing floating or gastroretentive dosage forms. This approach for metformin extended release tablet has been reported by Priyadarshini *et al.* based on a composite matrix of hydrophilic TSP and hydrophobic copolymer of polymethacrylamide-*g*-gellan. TSP was used as a release modulator cum-mucoadhesive polymer while buoyancy was contributed by sodium bicarbonate.¹³⁸ Ofloxacin floating tablets using TSP with pectin have also been reported recently.¹³⁹

Mali *et al.* have developed floating-bioadhesive sustained release tablets (12 h) for gastric retention of Verapamil by employing CMTSP, sodium bicarbonate, and HPMC. A similar concept of floating-bioadhesive sustained release drug delivery system was tried by Rajab *et al.* for Metformin HCL using TSP in combination of PVP or HPMC.^{140,141} Thiolation of TSP has been attempted with a consequent increase in its biomucoadhesive properties. The research group has done a comparative analysis of metronidazole containing thiolated TSP gel against the one containing plain TSP, wherein they concluded that thiolated TSP had 6.85-fold greater mucoadhesive strength. The studies were carried out using chicken ileum.¹⁴²

Simvastatin has an absorption window in the gastric region. Though CMTSP has an excellent mucoadhesive nature it was further converted to its thiomers by conjugating it with an amino acid; cysteine in the presence of a coupling agent to prolong the localization of the DDS at the target site. This is because thiolated polymers are able to form covalent bonds with thiol-rich domains on mucus membrane and thus incorporate better mucoadhesion and are also believed to increase gastrointestinal permeability and withstand the peristaltic housekeeping wave. The research group concluded that mucoadhesive strength was a function of the thiomers concentration in the formulation. They were able to establish a 1 : 1 IVIVC (level A) as shown in Figure 16.3, F3 being best formulation.¹⁴³

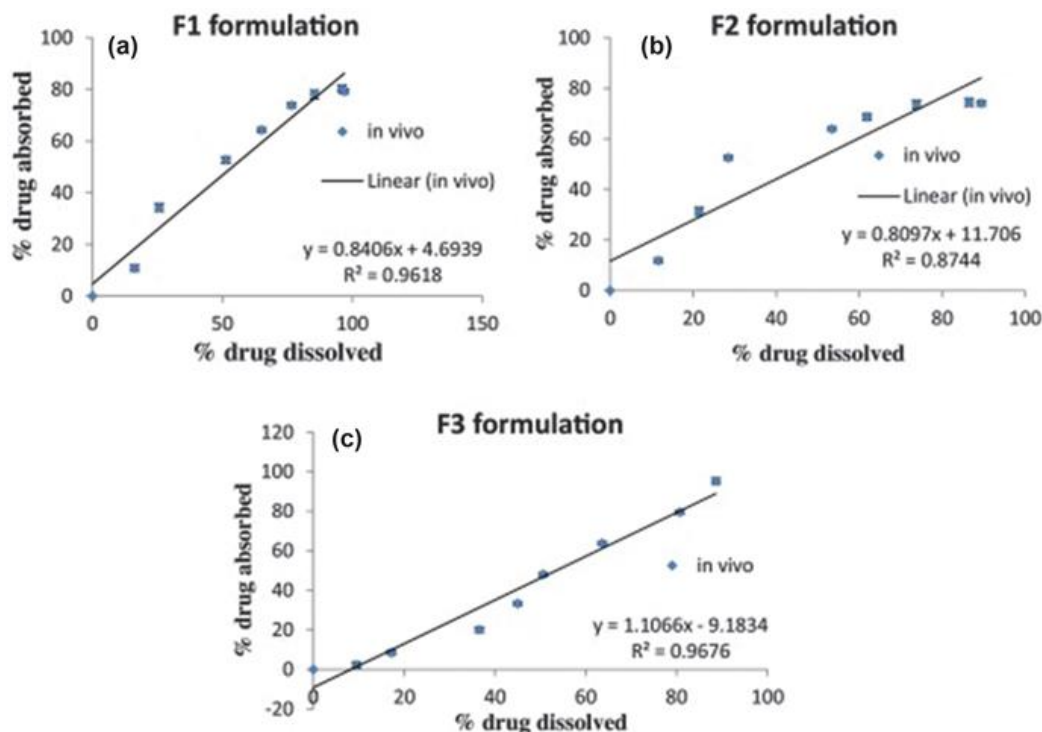


Figure 16.3 IVIVC for formulation: F1 (low concentration of thiomers), F2 (medium concentration of thiomers), F3 (high concentration of thiomers) (mean $\pm$ S.D.). Reproduced from ref. 143 with permission from Elsevier, Copyright 2016.

16.4.1.5 Ionic Gel/Beads/Interpenetrating Polymer Network (IPN)

Ion gels with improved viscosity, viscoelasticity, and thermal stability at room temperature and thixotropy were prepared using TSP and biobased and synthetic ionic liquids which were better as compared to hydrogels.¹⁴⁴

Nayak *et al.* have developed and characterized pH-sensitive TSP–alginate composite beads for controlled diclofenac sodium delivery.¹⁴⁵ Bera *et al.* further modified the release retardant system and developed alginate gel coated, olive oil-entrapped calcium alginate–tamarind gum–magnesium stearate composite buoyant beads for intragastric risperidone delivery to improve its oral bioavailability. These beads were prepared by ionotropic emulsion gelation technique to achieve prolonged delivery of this antipsychotic drug.¹⁴⁶

Application of TSP in the form of IPN microbeads was explored by Kulkarni and his co-workers along with sodium alginate¹⁴⁷ for controlling the release of Diltiazem HCl which was used as a model drug. Initially diltiazem-Indion 254[®] (a cation exchange resin) complex was prepared which was then entrapped within IPN microbeads prepared by ionotropic gelation. They were then covalently crosslinked to finally obtain dual crosslinked beads which released drug for 9 h which was confirmed by *in vivo* studies in rats. Scheme for the formation of IPN between TSP and sodium alginate is shown in Figure 16.4.

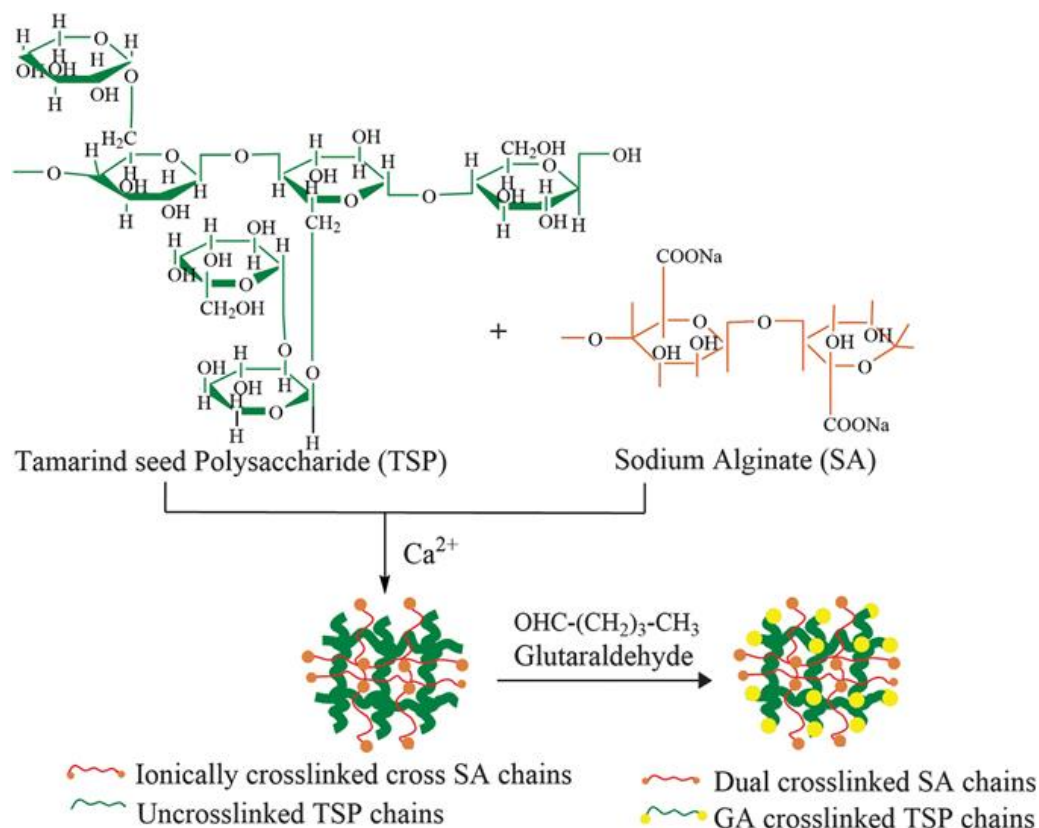


Figure 16.4 Schematic figure of the interpenetrated polymer network.

Nayak *et al.* in one study have blended TSP¹⁴⁸ gellan gum and then with low methoxy pectin¹⁴⁹ to produce sustained release mucoadhesive beads containing Metformin HCl. Both followed a zero order release pattern for 10 h. Using the property of ionotropic gelation of sodium alginate by calcium ions and combining it with the mucoadhesive and superior encapsulation properties of TSP, these two gums were combined in the ratio of 1 : 1 to prepare a multiparticulate system for sustained release of gliclazide. *In vivo* study revealed that gliclazide loaded TSP–alginate mucoadhesive microspheres produced a significant hypoglycemic effect on oral administration. Gliclazide solution administration showed a rapid reduction in blood glucose level up to 2 h and then rapidly came to normal levels. However, the reduction of blood glucose levels in the groups treated with gliclazide loaded microspheres was slower than the group treated with pure gliclazide as shown in Figure 16.5. Reduction in glucose level was sustained over 12 h.¹⁵⁰ They have also reported a combination of TSP–alginate based product for gastroretention of diclofenac¹⁵¹ and for pH sensitive microparticle beads of metformin HCl which followed a zero-order controlled-release pattern with super case-II transport mechanism.¹⁵²

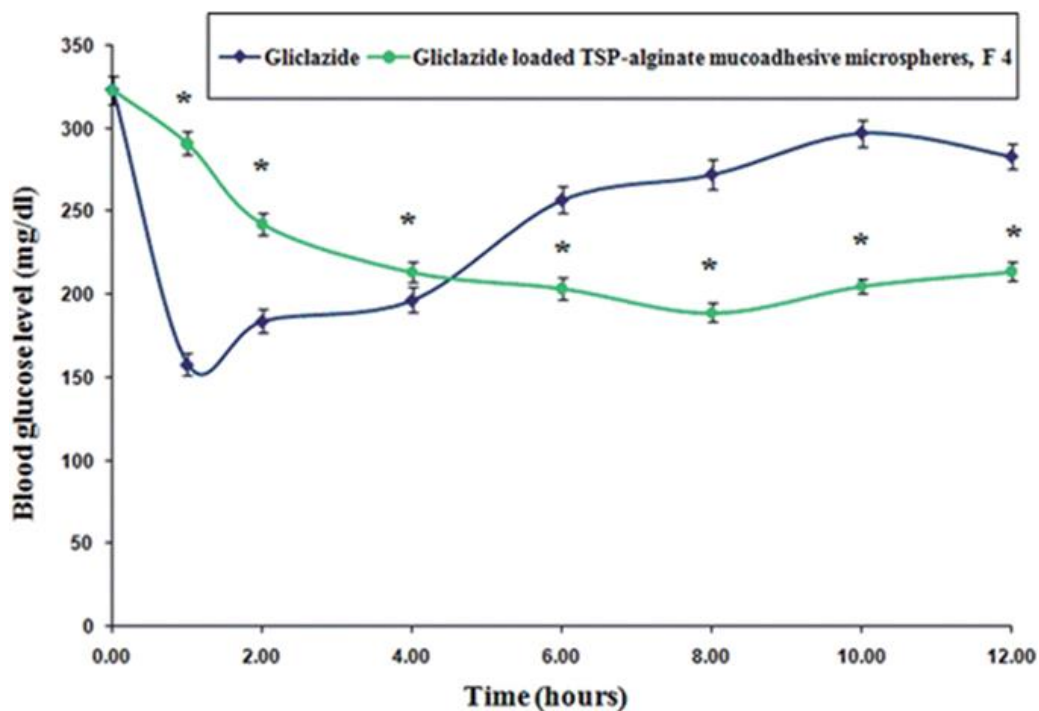


Figure 16.5 Comparative *in vivo* blood glucose level in alloxan induced diabetic rats after oral administration of pure gliclazide and gliclazide-loaded TSP–alginate mucoadhesive microspheres (F4) (mean $\pm$ SD, $n = 3$). Reproduced from ref. 150 with permission from Taylor and Francis, Copyright 2012.

CH and CMTSP in various ratios were used to develop an interpolymer complex film for colonic delivery of Budesonide, a drug used in IBD. This film was coated on budesonide tablets which were then evaluated for various parameters. It was concluded by them that NH_3^+ groups of CH form a stable complex with COO^- groups of CMTSP to form a film. Based on pharmacokinetic and pharmacodynamic evaluation a 40 : 60 ratio of CH : CMTSP proved to be the best of all combinations.¹⁵³ Jana *et al.* have incorporated glutaraldehyde cross-linked CH and TSP IPN for formulating microparticles for prolonged release of aceclofenac for 8 h which fitted to the Korsmeyer–Peppas model with a non-Fickian diffusion release mechanism.¹⁵⁴ The same concept was applied for aceclofenac¹⁵⁵ and also acyclovir¹⁵⁶ using gelatin and sodium alginate respectively instead of CH as compared to their earlier work. With aceclofenac IPN biocomposites they were able to produce anti-inflammatory activity in rats for 7 h. Similarly for acyclovir *in vitro* release was achieved for 7 h, with the ratio of alginate and CMTSP playing a vital role.

Combination of TSP and sodium alginate in the form of IPN (cross linked by glutaraldehyde) has been attempted for Propranolol HCl as well as propranolol–resin complex where sustained release was obtained for 24 h for the resinate.¹⁵⁷ Another strategy was used by Dey and co-workers where they developed a novel pH sensitive polymer by combining TSP, acrylic acid (AA), and polyamidoamine (PAA) by varying their ratios which was used for controlled release of 5-aminosalicylic acid (5-ASA) for 24 h. Controlled release kinetics of 5-ASA in simulated body fluid showed Fickian diffusion behavior. PAA acted as a carrier material for 5-ASA and simultaneously as a crosslinker, whereas AA was used to form semi-IPN and to impart pH responsive behavior to the materials.¹⁵⁸

Using the advantages of CMTSP, Mali *et al.* have cross linked CMTSP with glutaraldehyde to prepare aceclofenac loaded IPNs of CMTSP and CH to have sustained release of aceclofenac up to 12 h. The EE of hydrogels was found to be directly proportional to the concentration of CMTSP and crosslinker.¹⁵⁹ They have repeated this by cross linking TSP/CMC with citric acid to produce its hydrogel films to improve its release retardant properties for a model drug; moxifloxacin hydrochloride. CMTSP : citric acid was used in the ratio of 1 : 0.2 while in another study citric acid cross-linked CMC and TSP have been used for the same drug. The formation of crosslinks between citric acid and CMTSP were due to the esterification reaction between the two. The degree of cross linking, curing time, and temperature had a role to play in deciding the rate and quantity of drug release from the hydrogel film.^{160,161}

16.4.2 Applications of TSP in Futuristic NDDS

Besides NDDS, which have either been in the market or under research for many decades there are several upcoming areas where TSP has promising applications which we have reviewed herein separately.

16.4.2.1 Transdermal/Topical

A transdermal patch consisting of TSP loaded with 1% clindamycin and various ratios of glycerine and propylene glycol as the plasticizer and penetration enhancer respectively were prepared for acne treatment. A ratio of 4 : 6 showed the highest drug release and best efficiency in the antibiotic test and also proved that TSP could provide controlled release in a transdermal drug delivery system.¹⁶²

Polymeric nanofiber patches have evolved as one of the attractive DDS for skin diseases because of their several advantages, such as high ratio of surface area to volume, skin coverage, high porosity, and aspect ratio. Application of PVA and TSG composite patches has been explored by various research groups due to their highly viscoelastic nature, biocompatibility, and bioadhesivity.¹⁶³ Clindamycin-loaded PVA/CM TSP nanofiber patches were developed for acne using electrohydrodynamic atomization. Effects of various process parameters on the physiochemical properties of the product were examined. Antimicrobial activity of the drug-loaded PVA/TSP (9 : 1 ratio) patches was found to be significantly greater than that of the 1% clindamycin gel.¹⁶⁴

Another research group has loaded moxifloxacin into alginate beads as opposed to the films, to form a spongy wound dressing made of pectin/CMTSP for wound healing (Figure 16.6). Around 85% drug release was obtained from this dressing made of CMTSP : pectin (1.5 : 2) which showed excellent water vapor transmission and antibacterial activity. Application of this novel dressing was to reduce the frequency as well as pain which occurred during dressing change, each time. Bead loaded dressing allowed slow release of the drug at the wound area. Freeze drying was carried out to make the dressing spongy so as to absorb exudates of wound and for better water vapor transmission, for faster wound healing.¹⁶⁵

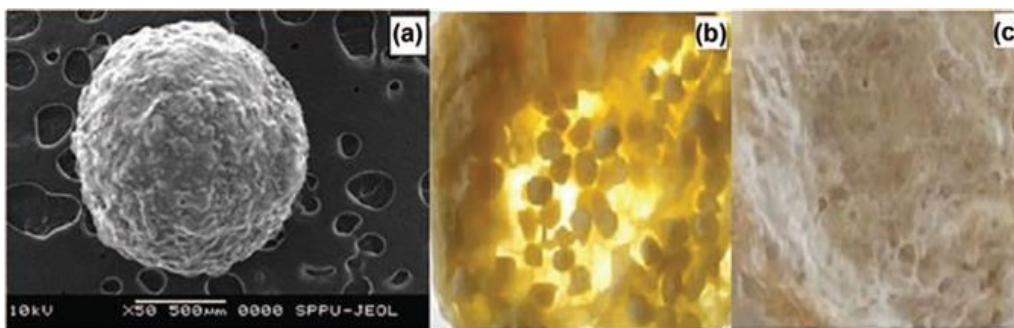


Figure 16.6 Scanning electron microscopy of moxifloxacin beads (a) 50 × magnification, (b) distribution of beads in wound dressing, and (c) porous wound dressing. Reproduced from ref. 165 with permission from Elsevier, Copyright 2019.

In one of the latest reports Malviya *et al.* developed a composite transdermal film for the delivery of a model protein molecule, human serum albumin; utilizing the film forming ability of CH and TSP. Factorial design (3^2) was employed to optimize the formula. Drug permeation studies were carried out for 6 days wherein around 78% drug permeated through egg membrane, and drug release following Hixson–Crowell kinetics.¹⁶⁶

16.4.2.2 Injectables/Implants

Suisha *et al.* have evaluated the application of enzyme degraded TSP for the intraperitoneal administration of mitomycin C, an antineoplastic antibiotic for effective control of the local lesion in the peritoneal cavity. Partially degraded by β -galactosidase, TSP has a better thermoreversible gelling property¹⁰⁷ which has been exploited by this research group. Gel containing 1.5% w/w TSP resulted in a broad concentration–time profile for the drug in plasma as compared to pluronic F127.¹⁶⁷

TSP and Eriochrome Black T (EBT) an antiangiogenic compound were combined into an implantable gel system, where increasing concentrations of EBT were able to give increased viscosities. To determine the release kinetics of EBT, release data were fitted to the Korsmeyer–Peppas model as shown in Figure 16.7, based on which it was found to give a sustained release profile following anomalous transport at 1.3% EBT and Fickian at 2.5% concentration thus showing dissimilar release mechanisms. EBT–TSP systems as per the authors appear to be suitable injectable implants for sustained delivery of EBT at the site of application, for the treatment of solid malignant tumors.¹⁶⁸

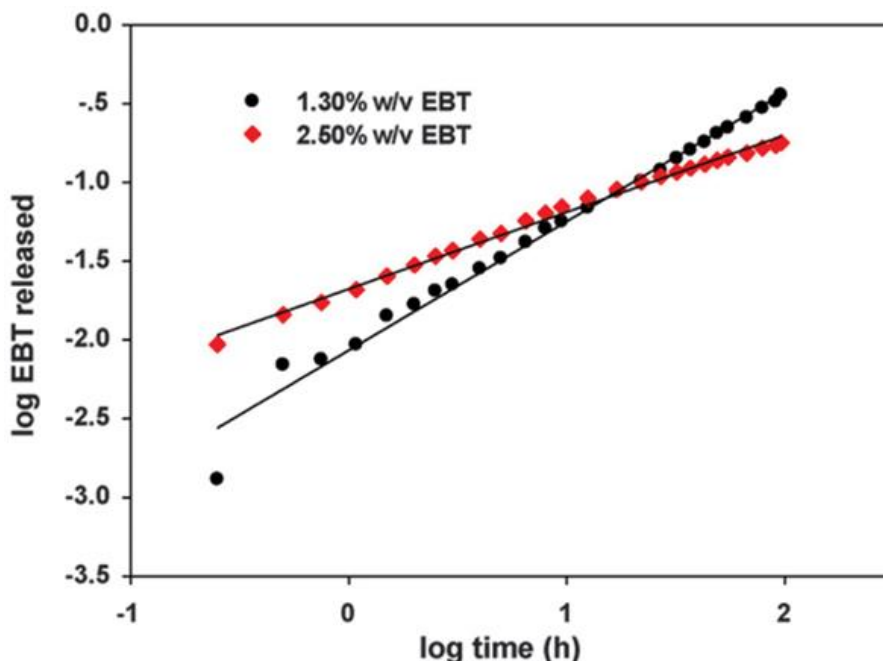


Figure 16.7 The plot of $\log(M_t/M_\infty)$ versus log time of 1.30% and 2.50% (w/v) EBT in 1% (w/v) TSX at 37 °C for the fitting using Korsmeyer–Peppas. Reproduced from ref. 168 with permission from Elsevier, Copyright 2010.

Hyaluronic acid as an intra-articular injection is an accepted treatment regimen for knee osteoarthritis. In order to search for a better and natural substitute, Yoon *et al.* combined HA with TSP for intra-articular injection prompted by its increasing use in ophthalmic delivery.⁸⁷ They evaluated these formulations for rheology, efficacy, and its lubricant activity against arthritis. The addition of TSP to HA stabilized the formulation against the effects of sterilization. It improved the outcome based on arthritis induced rat models, thus concluding that TSP can be considered as a substitute for HA or in combination with HA.¹⁶⁹

16.4.2.3 Hydrogels

Hydrogel matrices have been explored using TSP for various drug delivery applications including antimicrobial/antibacterial applications.¹⁷⁰ Composite cryogels of CMTSP and PVA have been prepared using freeze thaw-treatment with improved thermal stability of hydrogels. Metronidazole was used as a model drug which was released in a sustained fashion for 6 h. The ratio of CMTSP and PVA had a major role to play in the release profile and was seen to follow Higuchi release kinetics.¹⁷¹ Shaw *et al.* utilized protein–polysaccharide combinations, specifically gelatin and TG or CMTG for developing phase-separated hydrogels using ciprofloxacin as a model drug against *E. coli*. Physicochemical, thermal, and electrochemical characterization of the hydrogel along with its biological activity based on hemocompatibility and cell viability assay was carried out. Release kinetics of Ciprofloxacin loaded hydrogels and its antimicrobial activity were also studied. They concluded that the CMTSP-based hydrogel was better in many aspects, being viscoelastic and biocompatible, having excellent mucoadhesion, and pH-dependent swelling behavior. The study also documented higher proliferation of MG63 cells for CMTSP-based hydrogels as compared to the standard.¹⁷²

Ciprofloxacin loaded TSP-bentonite-based composite hydrogels were explored as ocular drug-delivery systems for antimicrobial activity. Most mechanically stable structure and maximum trans-corneal permeation of the drug was seen with a corresponding increase in bentonite proportion with TSP hydrogel release varying with the proportions of both.¹⁷³ Vyas *et al.* by using molecular dynamics and experimental evidence explored the capability of TSP supramolecular hydrogels to function as a matrix for holding hydrophobic oleic acid globules for an extended period of time to prevent their convergence and thus to be used as carriers for oil-based systems.¹⁷⁴

Aerogels have gained recent interest for biomedical applications due to their highly porous nature with low density and intrinsic properties. TSP was employed by Ghafar *et al.* for the preparation of composite aerogels by initially forming hydrogels by enzymatic oxidation of TSP with galactose oxidase and subsequently lyophilizing them to form aerogels. Nanofibrillated cellulose was incorporated by crosslinking into the TSP gel matrix. Synchrotron microtomography was utilized for the characterization of their microporous structure.¹⁷⁵

Building on the fact that partially degalactosylated^{107,176} TSP easily forms a thermoreversible hydrogel was put to use for the preparation of liquid formulations with *in situ* gelling properties for the oral delivery of cimetidine. They concluded that gelation of TSP does not require the presence of H⁺ ions and it can be used irrespective of the nature of the drug. Due to the fact that cellulose nanocrystals (CNC) can be converted to thermoresponsive hydrogels, Talantike *et al.* have combined these two by a green method to have degalactosylated TSP–CNC bioinspired hydrogel which forms a denser layer with tunable properties. Thermal transition from sol to gel occurred at 35 °C *i.e.* close to human body temperature.^{176,177}

Another group has investigated the effect of concentration of TSP (2.5–20% w/v in hydroethanolic solution) on the mechanical properties, stability, release retardant property, *etc.* on a series of moxifloxacin hydrogels. Drug release profiles from hydrogels indicated that the drug release increased with an increase in polysaccharide content as can be seen in Figure 16.8 where S5 represents the hydrogel with the highest amount of TSP.¹⁷⁸

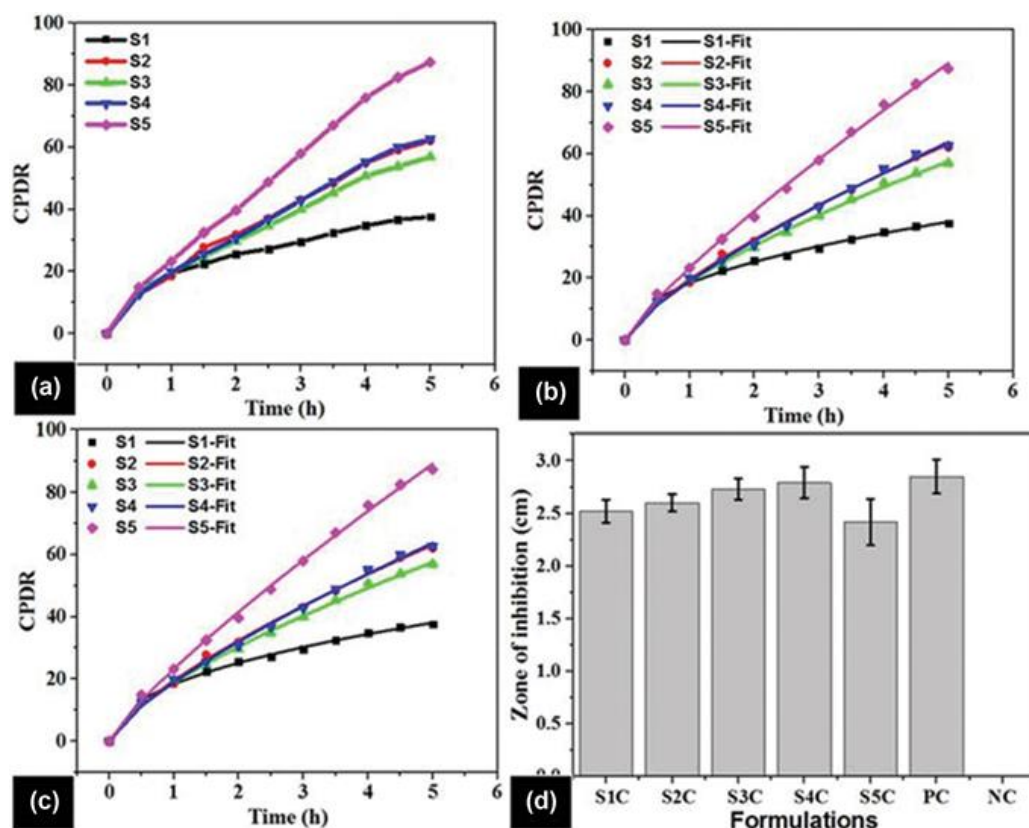


Figure 16.8 Drug release properties. (a) Cumulative percentage drug release (%) profiles, (b) Korsmeyer–Peppas model fitting, (c) Peppas–Sahlin model fitting, and (d) antimicrobial activity of the drug-loaded hydrogels. Reproduced from ref. 178 with permission from Taylor and Francis, Copyright 2018.

CMTSP blended diethanolamine-functionalized pectin (DFP)-based hybrid hydrogels reinforced with nanosized calcium silicate (CS) were developed for sustained delivery of a poorly soluble drug rutin. The dissolution profile of the optimized hydrogels (CMTSP : DFP : CS::3 : 7 : 1) followed a non-Fickian mechanism after fitting the drug release data into the Korsmeyer–Peppas kinetic model. These biopolymeric hydrogels also demonstrated smooth surface morphology, excellent mucoadhesion property, and biodegradability and have proved to be an attractive DDS for the delivery of rutin in its solubilized form.¹⁷⁹ In another study bio-aerogels based on TSP/XG and cellulose nanofibrils (CNF) in different ratios were developed by using a freeze-casting (– 20 °C for 1.5–2 h) procedure. Bio-aerogels showed high water absorption capacity along with shape recovery after compression, which was found to be a function of the electrostatic interactions, cross-linking, freezing conditions, and microstructure of the aerogels. After compression at 95% strain, the aerogel structure did not fracture but a denser structure was obtained at about 70–80% of the original thickness as shown in Figure 16.9.¹⁸⁰

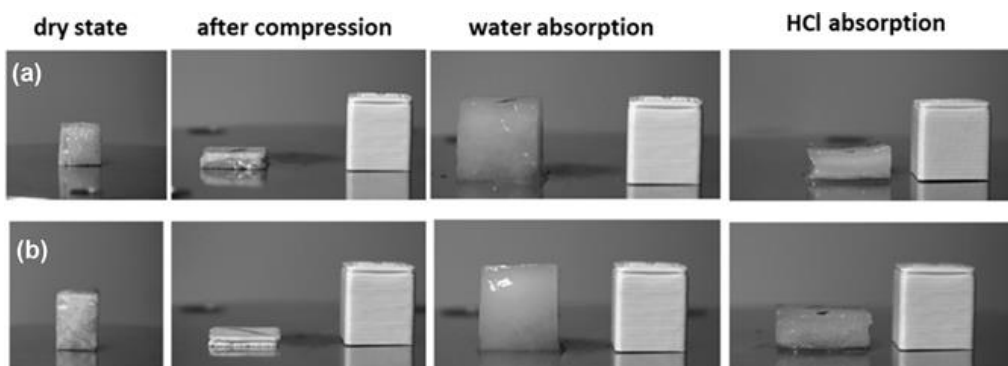


Figure 16.9 CNF aerogel (2%, UD freezing) at (a) 100 : 0 CNF/XG and (b) 60 : 40 CNF/XG in dry cubic shape, after compression, and after water absorption or after HCl 100 mM absorption. Cubic specimen in polylactide (PLA) on the right is used as the reference (CNF: cellulose nanofibrils; XG: xyloglucan; UD: unidirectional). Reproduced from ref. [180](https://doi.org/10.3390/gels7010005), <https://doi.org/10.3390/gels7010005>, under the terms of the CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

16.4.2.4 Nanoparticles/Nanoaggregates/Nanocomposites

Sustained release of the anticancer drug irinotecan has been proposed to minimize the adverse reaction of the drug in the body. With this aim mucoadhesive lyophilized nanoparticles (~ 400 nm) of this drug using TSP–sodium alginate (2 : 1) were developed using an ion gelation technique. An initial burst release within 1 h followed by sustained release up to 60% in 12 h was observed for this formulation.¹⁸¹ Continuing with their work on thiolated TSP Bhalekar *et al.* prepared acyclovir loaded lyophilized nanoparticles of thiolated xyloglucan using cysteine. The aim was to increase its permeability and hence bioavailability which was increased 2.57-fold with the TSP : cross-linker (sodium tri meta phosphate) in the ratio of 1 : 2.4.¹⁸²

Poly Electrolyte Complex (PEC) formation and a nanotechnology approach together has been used by researchers to improve the bioavailability of Simvastatin which has been found to have anticancer activity. Using an anti-solvent method various authors have brought about the interaction between the amino group of CH and the hydroxyl group of TSP leading to the formation of PEC stabilized crystalline cubic nanoparticles of Simvastatin which were self-organized, reversible, and formed by spontaneous association between oppositely-charged molecules without covalent cross-linkers. The anti-tumor activity of these nanoparticles was evaluated using human breast cancer cell lines (MCF-7). Initially tomato and egg-shell membranes were used to determine the drug release from different formulations where they had the ability to control drug release up to 24 h. Prepared cubic nanoparticles (53.3–383.1 nm) and specifically formulation T5 having the least particle size exhibited better control over the growth of MCF-7 than pure drug precipitates in *in vitro* conditions as shown in [Figure 16.10](#).¹⁸³

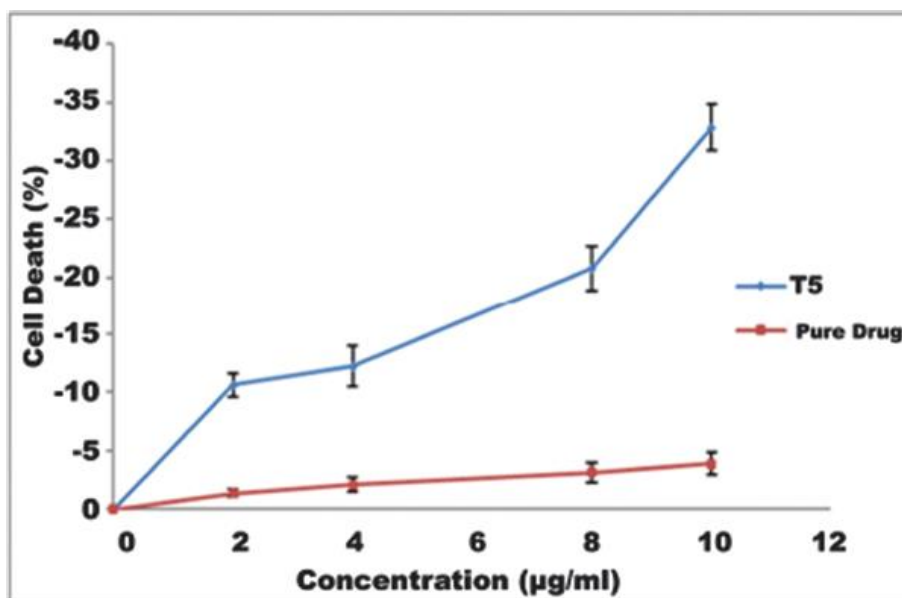


Figure 16.10 Anti-cancerous activity of nanoparticles (T5) and pure drug precipitates. Reproduced from ref. [183](#) with permission from Dove Medical Press, Copyright 2021.

Tamarind galactoxyloglucan–doxorubicin (DOX) nanoparticles were prepared *via* conjugation of DOX to TS galactoxyloglucan and subsequently ionic gelation with tripolyphosphate. This formulation possessed superior therapeutic efficiency due to the nano size and increased surface-to-volume ratio. They exhibited a pH-responsive DOX release in the acidic pH of 4.5, showing as high as 90% release in a sustainable manner with an aim to decisively deliver enhanced cytotoxicity against cancer cells while leaving normal cells unharmed.^{[184](#)}

Physical incompatibility of TSP with hydrophobic drugs makes it difficult to act as a nanocarrier for encapsulation and sustained delivery applications. Furthermore, being hydrophilic in nature it cannot self-assemble as individual entities in aqueous environments. To overcome these drawbacks, modification of physicochemical properties of TSP has been attempted by Dilbaghi *et al.* to render it amphiphilic in nature by introducing a hydrophobic group, octenyl succinic anhydride (OSA) onto its backbone ([Figure 16.2](#)). The resulting TSP–OSA conjugate was characterized using different techniques to assess its stability and suitability for novel drug delivery systems for designing nanostructures to encapsulate different types of drugs as self-assembled polymeric micelles.^{[51](#)}

TSP-based spherical compact nanoaggregates (60–140 nm) of camptothecin were prepared by a simple method in buffer solution which were able to release the drug for 6 h.^{[185](#)} They were also employed in combination with Poloxamer 407 for the formulation of tropicamide-loaded (1% w/v) ophthalmic nanosuspension for corneal permeation. TSP not only helped in providing mucoadhesive properties but it also significantly increased corneal permeation across the isolated goat cornea during *ex vivo* studies and improved EnE.^{[186](#)}

CMTSP-*g*-poly(*N*-isopropylacrylamide)-montmorillonite-based semi-IPN nanocomposites were synthesized and evaluated for their *in vitro* performance for lung cancer therapy. They were loaded with Erlotinib *via* probe sonication-assisted self-assembly protocol. Results implied an excellent uptake of these nanoscaffolds by the cells, demonstrating 97% EE and 99% drug release with outstanding mucin adsorption. These nanocomposites also demonstrated cytotoxic activity and induced apoptosis against A549 cells, which were significantly higher compared to plain drug.^{[187](#)}

Silver nanoparticles (AgNPs) have attracted use as antimicrobial agents due to their broad antimicrobial properties, but they suffer mainly from two drawbacks, cytotoxicity and stability. Successful application of AgNPs against bacterial infection needs development of improved and next generation AgNPs with better properties. Authors have used a unique “green-synthesis” approach and prepared CMTSP-capped AgNPs which have better properties than the currently available AgNPs for acting against bacterial biofilms. They had an average particle size of ~20–40 nm, showed long term stability, inhibited growth and biofilm formation of both Gram positive and negative bacterial strains and above all demonstrated reduced or no cytotoxicity against mammalian cells. They observed enhanced permeability of propidium iodide (a membrane impermeable dye) in CMT-capped AgNP-treated bacterial cells, suggesting that they may have caused membrane damage to cells which was confirmed by SEM Analysis (Figure 16.11).¹⁸⁸

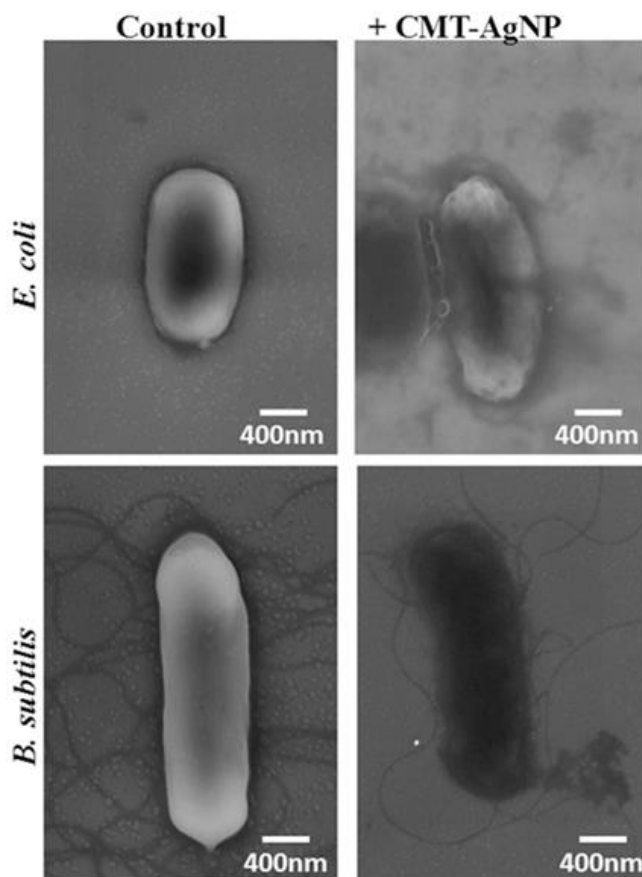


Figure 16.11 CMT-capped AgNPs cause bacterial membrane damage. SEM analysis of *E. coli* and *B. subtilis* cells treated with CMT-capped AgNP for 3 h and processed for SEM analysis. Scale bars correspond to 400 nm. Reproduced from ref. 188, <https://doi.org/10.1038/srep24929>, under the terms of the CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

16.4.2.5 As Graft Co-polymer and Composites

Microwave synthesis for preparing graft polymers has been attempted by some investigators. CMTSP-*g*-polyacrylonitrile graft copolymer was synthesized by Ahuja *et al.* They observed using response surface methodology that a large number of process variables were at play in deciding the

grafting efficiency and hence performance. These variables were microwave exposure time and power and the concentrations of acrylonitrile and ammonium persulfate. Results revealed higher thermal stability of graft copolymer, improvement in crystallinity, and pH-dependent swelling characteristics thus indicating its potential usefulness in designing pH-responsive DDS.¹⁸⁹

A combination of grafting and carboxymethylation was attempted by Badiger *et al.* Thermo-associating properties of two synthesized graft copolymers viz.; (i) carboxymethyl guar (CMG)-*g*-PEPO [PEPO = poly(ethylene oxide-co-propylene oxide)] and (ii) carboxymethyl tamarind-*g*-PEPO, (CMT-*g*PEPO) was determined. Grafting was executed by coupling reaction between amino terminated thermo-responsive PEPO with CMTSP using water-soluble ethyl carbodiimide hydrochloride (EDC) as a coupling agent. CMT-*g*-PEPO exhibited thermo-thickening behavior in water at 47 °C which was evidenced by rheological and fluorescence analysis. At moderate concentrations (10–30 g L⁻¹), the graft copolymer solutions showed soft gel-like natures. These thermo-associating polymers are non-toxic, bio-compatible, and thermo-thickening at a temperature near to physiological conditions, and can have potential applications as smart injectables in controlled drug technology and can also be used as thickeners in pharmaceuticals.¹⁹⁰

Trivedi *et al.* initially synthesized sodium salt of partially carboxymethylated TKP (Na-PCMTKP) followed by graft copolymerization of acrylonitrile onto Na-PCMTKP using ceric ammonium nitrate as a redox initiator. They got excellent swelling ability results from this hydrogel which can have application as an adsorbent material and in diapers as well.¹⁹¹ A novel pH-dependent hydrogel was synthesized by Ghosh *et al.* which comprised a TSP backbone on which was grafted polyacrylamide in the presence of microwave irradiation and an initiator. Effect of % grafting on the controlled release behavior of the model drug aspirin was evaluated. It was inferred by them that with an increase in percentage of grafting, swelling of matrices increased whereas erosion and rate of aspirin release decreased and that release followed zero order kinetics.¹⁹²

Mahajan *et al.* reported on the formation of new polymeric micelles by grafting Poly lactic acid (PLA) on xyloglucan. This novel amphiphilic co-polymer was synthesized by grafting of xyloglucan with PLA by ring opening polymerization reaction. Distinct properties of these micelles with a size of 102.4 nm were used for investigating the delivery pattern of curcumin. These micelles were freeze dried and assessed for their inhalation performance and targeting potential to lungs by *in vitro* and *in vivo* methods suggesting deep and prolonged residence of curcumin within lungs after pulmonary administration for effective treatment of lung cancer.¹⁹³

TSP has been copolymerized with PMMA and the properties of this graft copolymer have been tailored by adjusting the concentration of the reactants. This study was undertaken to produce a water-soluble material, with improved thermal stability and shelf life without affecting its hydrophilicity with potential to be used in DDS. Maximum percent grafting (84.7%) was achieved at 30 °C after 2 h. Formation of this graft co-polymer was evidenced by FTIR and NMR spectroscopy, differential scanning calorimetric studies, thermal gravimetric analysis, and scanning electron microscopy.¹⁹⁴

Graft copolymers with improved mucoadhesive characteristics have been synthesized by grafting of *N*-vinyl-2-pyrrolidone on TSP using microwaves. Maximum grafting was observed at lower microwave power for higher exposure time. Furthermore, buccal patches of metronidazole were formulated using this copolymer which provided prolonged bioadhesion during *ex vivo* studies, with more than 80% of the drug being released over a period of 6 h.¹⁹⁵

Taking advantage of its biocompatibility, pH non-sensitivity, stability, and non-toxicity TSP was combined with polyacrylamide to prepare a graft-copolymer to be used as a flocculating agent for a 2% w/v suspension of paracetamol having pseudoplastic behavior. A free radical method supported

with microwave irradiation was employed to synthesize polyacrylamide-*grafted*-tamarind seed gum using ceric(iv) ammonium nitrate as a free radical initiator.¹⁹⁶

With an aim of altering the basic structure of TSP to improve its functionality Deshmukh and co-workers hydrolyzed polyacrylamide grafted carboxymethylxyloglucan (HPam-*g*-CMXG) which was then converted into spherical microbeads using ferric ions as a cross-linking agent. They have explored its potential as a matrix material for intestinal targeted pH responsive microbeads for sustained delivery of the model drug aceclofenac. A correlation was established between process parameters and the performance of SR microbeads as well as its release mechanism.¹⁹⁷

A novel method for the synthesis of HAP composites in the form of blocks, laminates, and flower-balls by solvothermal regulation using TSP and Guar gum as modifiers was developed using Doxorubicin as a model drug for targeted drug delivery. Hydroxy apatite (HAP) crystals were assembled into porous spherical particles with gums acting as the core. Advantages were high drug loading capacity with very negligible cytotoxicity and biocompatibility for its application in targeted drug delivery. Maximum drug loading of DOX for HAP materials prepared by polysaccharide modification was 236.18 mg g⁻¹ along with prolonged release.¹⁹⁸

16.4.3 Biomedical Applications of Tamarind Seed Polysaccharide

Researchers around the world have reported the use of tamarind seed polysaccharide in various biomedical applications mainly for tissue engineering which includes skin tissue regeneration, bone regeneration, wound healing, etc.^{63,199} This is due to its good water absorbing and retaining ability.²⁰⁰ In this chapter, besides its role in a plethora of NDDS as seen above we also present scientific evidence that supports the use of TSP for several biomedical applications which have been summarized in Table 16.4.

Table 16.4 Summary of biomedical applications of TSP.

Area of biomedical application	Formulation details	Therapeutic actives	Research findings	Ref.
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Wound dressing material in wound healing	Carboxymethylcellulose and tamarind gum (CMC–TG) hydrogel dressing	Vancomycin	✓ Potential material for wound dressing	204
	Novel wound dressing using extracted TSP	Povidone iodine	✓ Rapid epithelialization and faster wound healing	205
	TX–PVA hydrogel films with sensors	—	✓ Wireless smart wound dressings approach	206–208
	TX–PVA		✓ Supporting material for skin tissue regeneration.	
	<i>C. odorata</i> extract loaded CMTSP coated cotton gauze	<i>C. odorata</i> extract	✓ Better bactericidal activity	209
	Xyloglucan–sugar hydrogel	—	✓ Effective in deeper wounds	210
	Sprayable film forming system	Propolis	✓ Good antibacterial activity	211

Tissue regeneration/engineering, cell therapy and cell adhesion	Xyloglucans for proliferation of skin cells	—	✓ Improved skin cell proliferation	213
	Hydroxyethyl methacrylate grafted CMTSP matrix scaffold	—	✓ Biocompatible hydrogel matrix	214
	TSP–XG for ophthalmic use	—	✓ Improved corneal wound healing	215
	Gelatin–TSP/CMTSP films	Ciprofloxacin	✓ Proliferation of human keratinocytes (HaCaT cells)	216
	Nanostructured cellulose–gellan–xyloglucan–lysozyme dressing	Mesenchymal stem cells	✓ Improved collagen deposition in deep burns	217
	PVA–CMTSP–graphene oxide (GO) composite films	Ciprofloxacin	✓ Altered keratinocytes proliferation	219

Bone tissue regeneration and cartilage tissue engineering	Injectable <i>in situ</i> gels of partially degalactosylated xyloglucan for cartilage reconstruction	Growth factor FGF-18	✓ Strong affinity of growth factor towards the polymer	222
	Xyloglucan-co-methacrylic acid/hydroxyapatite/SiO ₂ nanocomposite scaffold	—	✓ Improved osteoblast cell attachment	223
	Hydroxyethyl methacrylate grafted hydrogel of CMTSP	—	✓ Effective adhesion and growth of bone precursor cells	224
	Hydrogel of tamarind kernel powder, grafted by acrylic acid (TKP-AA)	—	✓ Mineralization of osteoblasts in Saos-2 cells	225
	TSP-chitosan capped gold nanoparticles	—	✓ Supportive matrix for the growth of hydroxyapatite	226
	Nano-hydroxyapatite/chitosan-tamarind seed polysaccharide nanocomposites	—	✓ Increased biomineralization	227

Neural tissue engineering	Thermo-responsive hydrogel scaffolds, sol-gel system of functionalized xyloglucan	Poly(α -lysine)	✓ Survival of neural stem cells on the functionalized scaffold	229, 230
			✓ Axonal penetration <i>in vivo</i>	
	Thermo-responsive xyloglucan hydrogel composite scaffold	Poly(L-lactic acid), glial-derived neurotrophic factor (GDNF)	✓ Sustained delivery of GDNF	231
			✓ Improved milieu of the injured brain	
Liver tissue regeneration	Xyloglucan containing galactose as the extracellular matrix	Extracellular matrix	✓ Enhanced spheroids formation in hepatocytes	234–236
	Alginate microcapsules with xyloglucan (AL/XG) extracellular matrix	Extracellular matrix	✓ Rapid formation of spheroids in hepatocyte	235, 236
Diagnosis	TSP-based FITC biopolymer for theranostic management of cancer	—	✓ Time dependent uptake in tumor microenvironment	237
	Magnetic nanocapsules of TSP loaded with hydrophilic sulfated quercetin	—	✓ Improved drug delivery in cancer treatment	238

16.4.3.1 TSP as Wound Dressing Material in Wound Healing

Wound healing and reepithelization depend upon the re-establishment of dermis and epidermis layer of skin. Hydrogel-based wound dressings are popular for healing a variety of wounds as they protect

the wounds from microbial and environmental factors.^{201–203}

Mali *et al.* used carboxymethylcellulose (CMC) and tamarind gum (TG) together for the preparation of hydrogel wound dressings and developed CMC–TG based citric acid cross-linked vancomycin-loaded hydrogel film for wound healing.²⁰⁴ The CMC–TG hydrogel dressing showed pH-dependent swelling, with maximum swelling being observed for the 1 : 1 ratio. They also observed a significant decrease in swelling with an increase in the concentration of TG and cross-linker along with controlled release of vancomycin until 24 h.

Patil *et al.* fabricated novel wound dressing films using extracted TSP and further crosslinked with epichlorohydrin in the presence of sodium sulfate. Optimized TSP films were further loaded with povidone iodine (PI) by using a soaking technique.²⁰⁵ It was observed that the elasticity of the TSP film depended on the thickness of film and degree of cross-linkage. Results of the swelling study indicated that all the films remained intact without dispersing in swelling media with good swelling properties which is essential to absorb the exudates from the wound, thereby, keeping the wound dry which also promotes the healing process. Rats treated with PI loaded TSP films showed rapid epithelialization and higher collagen content. Thus use of TSP for wound–healing properties was proved as they improved processes of re-epithelialization and remodeling.

Ajovalasit *et al.* synthesized TX-based hydrogel films along with polyvinyl alcohol and glycerol which was further crosslinked with glutaraldehyde.²⁰⁶ The absence of cytotoxicity as well as good physical and mechanical properties of synthesized TX–PVA hydrogel films make them appropriate candidates for combination with sensors to observe the wound healing mechanism and biological investigations in animal models.

Ajovalasit *et al.* in continuation with their earlier research developed a TX–PVA hydrogel membrane crosslinked with glutaraldehyde for wireless smart wound dressings suitable to integrate sensing and communication platforms for remote monitoring.²⁰⁷ It was observed that the developed dressings absorb simulated body fluids, without losing their integrity with significant microstructural changes. Increase in electric conductivity and permittivity with swelling degree was observed which confirms that the developed films can be further exploited for sensing applications. The authors concluded that an increase in conductivity is due to the increase of mobility of residual sodium and chlorine ions from the synthesis. This observation suggests the possibility of pre-loading the hydrogel with small amounts of ions with various charge densities and mobilities to modulate the impact of water uptake on the electromagnetic properties of the film, and in turn the response of the RFID antenna.²⁰⁷ A parallel biological study on the same hydrogel films demonstrated that they are non-cytotoxic, hemocompatible, non-adhesive to the wound, and able to provide protection from bacterial infiltration. Picone *et al.* developed homogeneous, slightly opaque XG–PVA hydrogel films crosslinked with glutaraldehyde. They showed partial adhesion to cells thus supporting cell growth and contributing to skin tissue regeneration. High retention of aqueous fluids and absorption of serum proteins by TX–PVA film is seen which suggests that the developed film can grant a moist wound environment while absorbing exudates. The film was found to be cyto-compatible, blood-compatible, and did not show any immunogenic response.²⁰⁸

Based on the work carried out by Sittikijyothin *et al.*²⁰⁹ TSP exhibited wound healing activity by improving the liquid absorption ability when it was used as a coating material of cotton gauzes loaded with *Chromolaena odorata* leaf extract to achieve antibacterial along with hemostatic activity. Blood clotting effects of *C. odorata* extract indicated that it had excellent hemostatic efficacy mainly in abrasions and lacerations. They concluded that the amount of CMTSP affected the physical properties and absorption capacity of the *C. odorata* extract loaded CMTSP coated cotton gauze. It exhibited better bactericidal activity against *S. aureus* than against *E. coli*. Xyloglucan–

sugar hydrogel and sheets devoid of any drug were developed for deep wound healing. Sheets made from a mixture of 3.0% (w/v) TSX solution and 33.3% (w/w) sucrose, displayed enough strength, wound surface status, and water content, when compared with Xyl/Suc (1 : 2) hydrogel and a commercial gauze and polyurethane film, using a rat deep wound model caused by serious frostbite.²¹⁰

A very interesting application of TSP has been delineated in one study where a natural film forming system has been developed of propolis extract for its wound healing properties. CMTSP was incorporated as against TSP as it improves cold water solubility and viscosity and also increases swelling rate. Sting-free bee-derived propolis was added as an active for its anti-inflammatory, antiproliferative, antimicrobial, and antioxidant activities. Glycerin/PEG was used as a plasticizer. This formulation (propolis extract: 250 $\mu\text{g ml}^{-1}$) was also able to inhibit the growth of *S. aureus* and *S. epidermidis*.²¹¹

16.4.3.2 TSP for Tissue Regeneration/Engineering, Cell Therapy, and Cell Adhesion

Hydrogels provide a 3D environment and are found to be useful as alternative cell carriers or scaffolds in cell-based regenerative therapies and for enhancing cell growth and tissue regeneration.²¹² Nie *et al.* investigated *in vitro* effects of TSP–xyloglucans on proliferation, migration, cell viability, cell cycle, and signal pathways in addition to their interaction with cells and so in promoting skin regeneration. Two methods were utilized *i.e.*, copper complex precipitation and a cold-water method for isolation of two xyloglucans from tamarind gum powder.²¹³ The extracted xyloglucan differed in molecular weight, rhamnose content, and ratios of xylose, arabinose, galactose, and glucose. It was observed that both the xyloglucans had an impact on cell proliferation and cell migration which helped in skin regeneration. Additionally, no inhibition or homeostasis of cell physiologic activities was detected.

Development of novel and healthy skin prepared through tissue engineering applications is gaining attention by researchers due to variation in patho-physiological conditions of the skin in different conditions such as burns, skin ulcers, atopic dermatitis, psoriasis, *etc.* Choudhury *et al.* synthesized hydroxyethyl methacrylate grafted a carboxy methyl tamarind (CMT-*g*-HEMA) polysaccharide matrix scaffold for skin tissue engineering which can be suitable for clinical and commercial application in different skin disorders.²¹⁴ Authors proved that the developed hydrogel matrix is biocompatible with HaCaT, NIH-3T3, and mouse dermal fibroblasts. It was proved to be safe and devoid of cytotoxicity through a mitochondrial functionality assay.

The findings of research carried out by Burgalassi *et al.* suggest benefits of TSP in corneal epithelium wound healing.²¹⁵ Researchers checked the role of TSP–XG on the integrin–substrate recognition system and in repairing corneal wound. The presence of a mucin-like structure and strong mucoadhesive properties of TSP–XG supports the conjunctival cells and promotes corneal wound healing. TSP–XG improves adhesion of cells to laminin due to its greater interaction with the integrin recognition system than hyaluronate and helps in rabbit eye wound healing. In one study Shaw *et al.*²¹⁶ developed gelatin–tamarind gum (TKG)/carboxymethyl tamarind gum (CMTKG) phase separated films for skin tissue engineering purposes. The research findings revealed that increased hydrophilicity of CMTKG is responsible for less agglomeration in the CMTKG films as compared to TKG films. The authors further developed ciprofloxacin-incorporated films using the same composition of polymers which were found to be effective against *E. coli* with improved proliferation of human keratinocytes.

Recently Costa de Oliveira Souza *et al.*²¹⁷ investigated the use of a nanostructured cellulose–gellan–xyloglucan–lysozyme dressing seeded with mesenchymal stem cells (MSC) as the wound

dressing in a preclinical model for the treatment of deep second-degree burns. Modification in the hydrophilicity of membranes was reported due to inclusion of xyloglucan and carboxyl and amine groups which made the membranes hydrophobic which is favorable for MSC adhesion, proliferation, and differentiation. The authors reported reduced inflammatory infiltration with accelerated resolution of the inflammatory process, decreased injury area, increased vascular proliferation, improved collagen deposition and distribution, and complete epithelialization in 30 days. In another study TKP was modified by the monomer, acrylic acid (AA), in different mole ratios. It was found that TKP : AA (1 : 5) offers an appropriate surface for skin tissue engineering with sensory functions applicable *in vitro*, *in vivo*, and *ex vivo*. This combination showed excellent adhesion property and enhanced cell viability with no toxicity.²¹⁸

PVA–CMTG–graphene oxide (GO) composite films were developed to synergize the properties of CMTG and GO in reinforcing the properties of PVA films. Release and antimicrobial properties of Ciprofloxacin which was used as a model drug were studied. It was established that the prepared films alter cell proliferation of human skin keratinocytes in a composition-dependent manner and the films can be used for tissue engineering applications.²¹⁹

16.4.3.3 TSP in Bone Tissue Regeneration and Cartilage Tissue Engineering

Development of biomimetic material which supports bone tissue regeneration is a major challenge in the field of tissue engineering.^{220,221} Literature suggests the use of TSP–XG for bone tissue engineering applications due to its controlled swelling ability, biocompatibility, and biodegradable properties. Dispenza *et al.*²²² developed injectable *in situ* gels of a temperature responsive partially degalactosylated xyloglucan along with the growth factor FGF-18 for cartilage reconstruction. The developed gel was further investigated for injectability; the shear viscosity, gelling time at body temperature, morphology, porosity, and mechanical properties which are tunable by varying the polymer concentration. Growth factor shows a strong affinity towards the polymer and competes with it in the gelation process. Data of preliminary biological evaluations confirm an increase in cell proliferation with an increase in the concentration of FGF-18 protein and absence of cytotoxicity.

Research documents the use of (xyloglucan-co-methacrylic acid/hydroxyapatite (HAP)/SiO₂) polymeric nanocomposite scaffolds for bone tissue engineering applications.²²³ Researchers synthesized nanocomposite materials from free radical polymerization using n-SiO₂ and n-HAP–XG and methacrylic acid (MAAc) and further developed scaffolds by freeze drying method. Increasing the n-SiO₂ polymers in grafted XG-based biomaterials supported osteoblast cell attachment with adequate cell viability and cell growth. Research findings indicate that the developed nanocomposite scaffold is a potential biomaterial for bone tissue engineering.

A chemically-grafted hydrogel network of CMTSP with hydroxyethyl methacrylate in the ratio of 1 : 10 was constructed by Sanyasi *et al.*²²⁴ Research findings proved the suitability of the developed hydrogel for effective adhesion, growth, and further clustering of bone precursor cells viz. RAW (264.7). The developed material was found to be compatible for growing HUVEC and Neuro2a cells demonstrating its potential in clinical applications in the field of bone tissue engineering. In another study Kumar *et al.* reported suitability of a hydrogel produced from Tamarind Kernel Powder, grafted by acrylic acid (TKP–AA) for bone tissue engineering *in vitro*. A Porous and interconnected surface of THE hydrogel is observed by SEM which allows the growth of both osteogenic and non-osteogenic cells. Enhanced differentiation and mineralization of osteoblasts without the addition of exogenous growth factors is reported in Saos-2 cells.²²⁵

Biswal *et al.*²²⁶ have reported the suitability of TSP–CH capped gold nanoparticles as a matrix for the growth of HAP. X-ray diffraction spectrum has shown Bragg reflections which are similar with the HAP and energy dispersive X-ray studies confirmed calcium/phosphate stoichiometric ratio of HA. The study supports the developed TSP–CH capped gold nanoparticles as a useful matrix for the growth of HAP.

Shakir *et al.*²²⁷ fabricated nanoengineered biocompatible nano-hydroxyapatite (n-HAP)/CH–TSP nanocomposites by a co-precipitation method for bone tissue engineering applications. Authors reported strong chemical interactions between the three components n-HAP–CH–TSP as compared to n-HAP–CH. Optimized nanocomposites exhibited greater swelling character, acceptable degradation, increased biomineralization in simulated body fluid, and greater protein adsorption as compared to other formulations. A greater non-toxic response with MG-63 cells and improved hemocompatibility was observed with n-HAP–CH–TSP indicating that these nanocomposites could be promising alternative biomaterials for bone tissue engineering.

16.4.3.4 TSP in Neural Tissue Engineering

Foremost challenges in neural transplanting in the brain are variable graft survival, lack of integration, and rebuilding of the patient's tissue mainly due to the non-conductive environment in the human brain which is unable to provide sufficient physical support to the graft.²²⁸ Damage in the brain due to trauma or injury can inhibit axonal regeneration. Biomedical materials may improve this situation by providing a suitable cellular microenvironment.

In one of their research studies Nisbet *et al.*²²⁹ discussed the functionalization of xyloglucan with positively-charged poly(L-lysine) (PDL) and further developed thermo-responsive hydrogel scaffolds for neural tissue regeneration of the spinal cord. Xyloglucan under 2D and 3D culture conditions was found to be non-toxic and supported neural cell growth and neuronal differentiation. Additionally, functionalization helped in controlling the cell diameter, number, migration and the neurite density, and direction of growth. *In vitro* interaction of neural stem cells (NSCs) was checked with the xyloglucan scaffolds. The findings of the study revealed the survival of NSC on a functionalized scaffold similar to that of the primary cortical neurons, supporting its usefulness in the treatment of spinal cord injuries.

In another study, Nisbet *et al.*²³⁰ checked the suitability of the developed sol–gel system of xyloglucan and xyloglucan-grafted-PDL hydrogels, for the regeneration of damaged axons of neural pathways in the central nervous system through their implantation in the rat's brain. Penetration of neuritis and astrocytes as well as cell adhesion to the surface of the scaffold was seen at higher concentration of grafted PDL. Timing of the astrocyte migration matched with neurite infiltration within the scaffolds, signifying that astrocytes facilitated this infiltration, possibly due to the secretion of laminin. The authors concluded that the functionalized xyloglucan hydrogels encouraged axonal penetration *in vivo*, providing direction to deal with future challenges.

Wang *et al.*²³¹ developed a thermo-responsive xyloglucan hydrogel composite scaffold, engrossed with electrospun PLA short nanofibers for bone tissue regeneration in the injured brain. A protein which promotes cell survival and axonal growth, glial-derived neurotrophic factor (GDNF), was further blended with the composite scaffolds for controlled delivery. They confirmed the ability of the scaffolds to support ventral midbrain dopamine progenitors, and also sustained delivery of GDNF to support cell survival and dopaminergic axon growth. Researchers concluded that the developed functionalized composite scaffolds provide a means to significantly improve the milieu of the injured brain.

16.4.3.5 TSP in Liver Tissue Regeneration

In the last few years substantial research has been directed toward the development of new natural and synthetic biomaterials for both culture and cell delivery which promotes liver regeneration.^{232,233}

Seo *et al.*²³⁴ checked the suitability of xyloglucan-containing galactose in the development of synthetic extracellular matrix (ECM) for adherence of the hepatocytes in tissue engineering. Adherence of 73.9% hepatocytes to a xyloglucan-coated polystyrene dish and 59.1% to non-coated polystyrene dish was observed. Specific interaction between galactose moieties of XG and asialoglycoprotein receptors of hepatocytes was seen. It has also been found that there is a decrease in DNA synthesis of hepatocytes attached to the XG-coated surface with an increase in the coating concentration of XG as well as in the presence of epidermal growth factor. They²³⁵ further developed alginate microcapsules with xyloglucan (AL/XG) as a synthetic ECM for hepatocyte attachment. A decrease in albumin secretion, ammonia elimination rates, and cell viability of hepatocytes with culture time in AL/XG capsules was seen, indicating rapid formation of hepatocyte spheroids in AL/XG capsules than in AL capsules. They further concluded that enhanced spheroid formation in hepatocytes in the presence of XG can act as a new synthetic ECM and can further enhance the liver-specific functions in three-dimensional (3D) space. Deng *et al.*²³⁶ used xyloglucan, a new synthetic ECM for HepG2 cell attachment in alginate capsules. They reported enhanced liver functions of HepG2 cells in the alginate/xyloglucan scaffold and provided support in cell adhesion, proliferation, and differentiation.

16.4.3.6 TSP in Diagnosis

TSP also finds application in diagnosis. Fluorescein isothiocyanate (FITC) a fluorescent probe is widely used for diagnostic and therapeutic purposes and for the synthesis of more photostable grafts. However, its solubility is reported to be less than 0.1 mg ml^{-1} in water and it also decomposes in aqueous solutions. Low-cost TSP-based FITC biopolymer has been synthesized by hydroxyl-carbamoyl interaction for studying both the polymer and dye for conjugation chemistry and bio-distribution studies. It was investigated for its chemical conjugation and cellular uptake efficiency in cancer cell lines as well as macrophages. Galactoxyloglucan exhibited a time-dependent uptake in a tumor microenvironment in both *in vitro* and *in vivo* studies. This can pave the way for theranostic management of cancer.²³⁷

Magnetic nanocapsules of TSP loaded with hydrophilic sulfated quercetin, a flavonoid was synthesized by an inverse miniemulsion process through interfacial polymerization. Polyacrylic acid sodium salt (PAAS) covered by magnetite nanoparticles ($\text{Fe}_3\text{O}_4\text{@PAAS}$) which produce an aqueous ferrofluid with superparamagnetic behavior was used here. At the end of the drug release study, 67% of sulfated quercetin was released. This would increase the efficiency of cancer treatment and thus represent a new perspective for biomedical applications, not only for drug delivery but also for magnetic hyperthermia and agent contrast in MRI.²³⁸

Nanotheranostics which involves simultaneous diagnosis and therapy using nanoparticles has been put to use to develop folate receptor targeted polysaccharide coated iron oxide nanoparticles to act as MRI contrast agent as well as an effective drug delivery vehicle for doxorubicin (DOX). Unnikrishnan *et al.* used the hydroxyl group of galactoxyloglucan, isolated from tamarind seed kernel which has proven to have immunomodulatory effects to develop folic acid conjugated galactoxyloglucan-iron oxide nanoparticles (FAPIONPs). DOX with proven antineoplastic activity has shown through other DDS to induce hypersensitivity reactions in treated patients. So to prevent

any systemic toxicity DOX–FAPIONPs were fabricated for the dual purpose of cancer diagnosis and therapy. From Figure 16.12 it can be confirmed that administration (i.p.) of DOX@FAPIONPs reduced the tumor weight to a larger extent while FAPIONPs presented no impact on tumor size.²³⁹

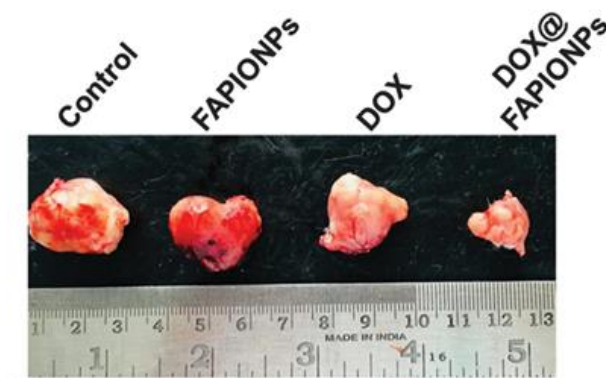


Figure 16.12 *In vivo* therapeutic efficacy in DLA solid tumor mice: representative photographic image of the tumors excised from the saline-treated control, FAPIONPs, free DOX, and DOX@FAPIONPs (DLA: Dalton's lymphoma ascetic) cells. Reproduced from ref. 239 with permission from Elsevier, Copyright 2021.

PST001 (galactoxyloglucan isolated from tamarind seed) is shown to be capable of anticancer, antiangiogenic, and anti-metastatic activities.²⁴⁰ Most of the magnetite iron oxide nanoparticle (IONP)-based drug delivery systems tend to produce hypersensitive reactions. Hence Unnikrishnan *et al.* fabricated doxorubicin-loaded carboxymethylated PST001-coated iron oxide nanoparticles (DOX@CM-PST-IONPs) for better management of cancer. PST001 was made to react with monochloroacetic acid to generate carboxymethylated PST001 (CM-PST) which bound to the metal surface of aminated iron oxide nanoparticles (IONPs) and conjugated with DOX to generate DOX@CM-PIONPs (Figure 16.13). These biocompatible and non-toxic nanoconjugates were found to be effective in both 2D and 3D cell culture systems with efficient cancer cell internalization. DOX@CM-PIONPs thus with additional and beneficial therapeutic effects exhibited potential to be used as a fluorescence probe for non-invasive imaging to monitor the cellular uptake and release of permeated doxorubicin. In murine models they showed greater therapeutic efficiency compared to clinically used DOX.

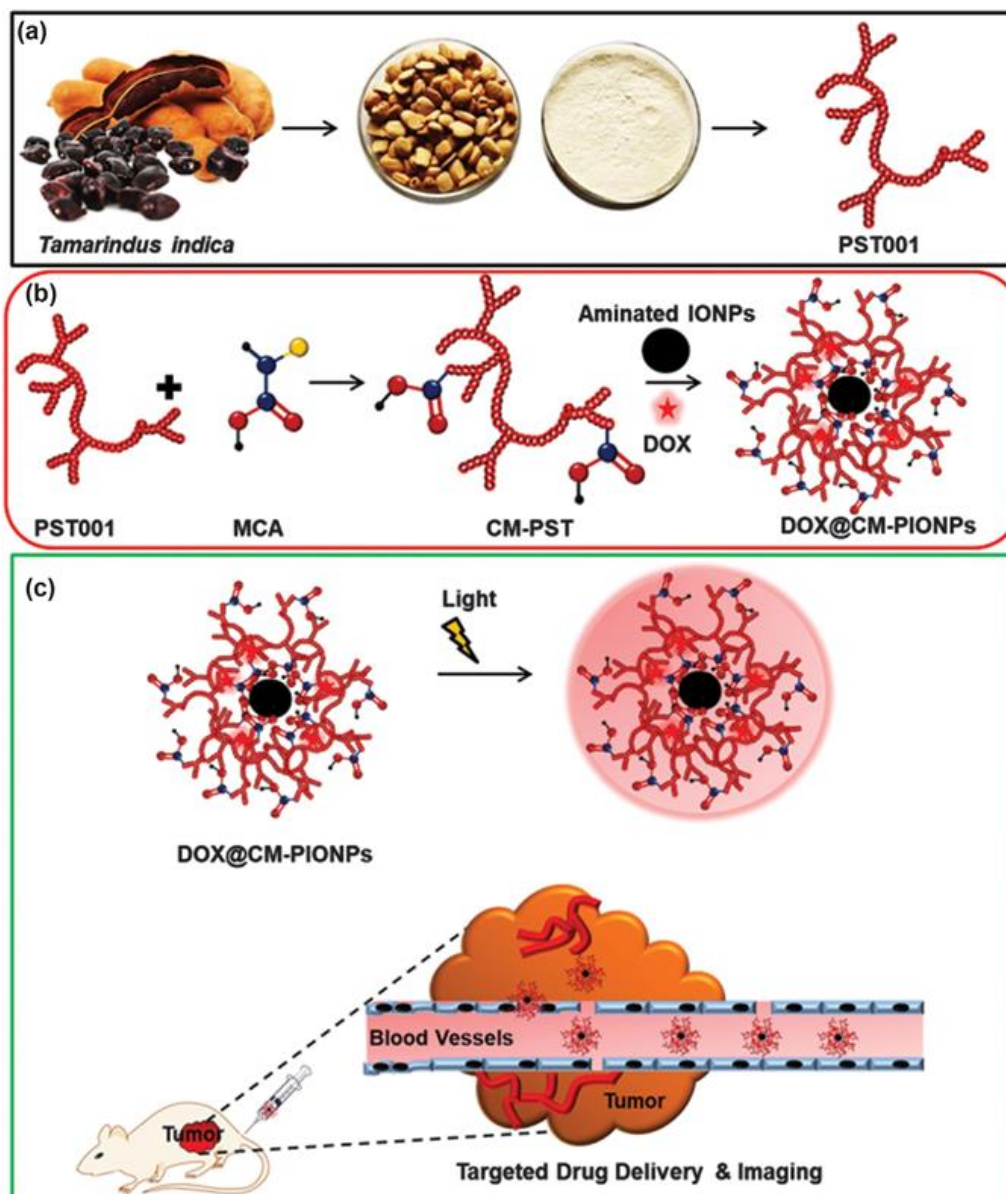


Figure 16.13 Steps involved in the production of biogenic DOX@CM-PIONPs. Reproduced from ref. 240, <https://doi.org/10.1038/s41598-021-87364-y>, under the terms of the CC BY 4.0 license <https://creativecommons.org/licenses/by/4.0/>.

Joseph *et al.*^{241,242} exploited the potential of PST001 for drug delivery and theranostic applications mainly by fabricating nanocomposites comprised of silver/gold nanoparticles with PST001. They developed nanochemobiotics, which are proven to have potential theranostic applications viz. chemotherapy in fusion with antibacterial efficacy for both Gram positive and negative strains with real time *in vivo* surface enhanced Raman scattering (SERS) monitoring for efficient management of bacterial infections and cancer. This multi-therapeutic nanoprobe, where PST001 was present as a capping layer over silver nanoparticles (SNP), enabled them to execute targeted cytotoxicity. While in another study using the same green approach PST001-capped AuNPs (PST-GNPs) with a cancer-cell-selective toxic nature and excellent biocompatibility were developed.

They were used to monitor real-time biodistribution in a solid tumor-bearing syngraft mice model proving to be an excellent biomedical tool as the PST-GNPs were able to act as a promising SERS substrate in exploring the accumulation of tumors.

16.5 Conclusion

Advances in biomedical technology and expansion of NDDS have given rise to newer excipients. Understanding and identifying novel uses of existing excipients is less expensive and less time-consuming than the development of newer excipients. Tamarind seed polysaccharide/gum/XG and its derivatives have been extensively exploited to be used as versatile and multifaceted excipients in conventional and NDDS, as well as in biomedical applications due to their suitable physicochemical, biological, and mechanical properties. This chapter has provided an overview of the research pertaining to tamarind seed polysaccharide not only as an extensively used polymer to date but has also emphasized its utilization as a prospective biomaterial/excipient in different DDS and biomedical applications. The budding research scholars, academicians, and entrepreneurs working on TSP-based DDS and their application in the biomedical field will definitely benefit from this framework. The authors hope that the multifunctionality and safety of TSP would definitely meet the regulatory requirements and that TSP-based DDS will serve the therapeutic needs of people at large in the future.

Abbreviations

TSP	Tamarind seed polysaccharide
GRAS	Generally recognized as safe
kDa	Kilo daltons
GC-MS	Gas chromatography-mass spectroscopy
2D NMR	2 Dimensional nuclear magnetic resonance
NMT	Not more than
EPA	Environmental protection agency
CMTSP	Carboxy methyl tamarind seed polysaccharide
EE	Entrapment efficiency
NDDS	Novel drug delivery system
BCS	Biopharmaceutical classification system
HPMC	Hydroxy propyl methyl cellulose
IOP	Intra ocular pressure
HA	Hyaluronic acid
DPI	Dry powder inhaler
PVA	Poly vinyl alcohol
<i>P. Aureogenosa</i>	<i>Pseudomonas aeruginosa</i>
<i>B. Subtilis</i>	<i>Bacillus subtilis</i>
<i>E. coli</i>	<i>Escherichia coli</i>
SEM	Scanning electron microscopy
DSC	Differential scanning calorimeter
MCC	Microcrystalline cellulose
NaCMC	Sodium carboxy methyl cellulose
STMP	Sodium trimetaphosphate
DDS	Drug delivery system

PVP	Poly vinyl pyrrolidine
IVIVC	<i>In vitro in vivo</i> correlation
IPN	Interpenetrating polymer network
IBD	Irritable bowel disease
EBT	Eriochrome black T
CNC	Cellulose nanocrystals
DFP	Diethanolamine functionalized pectin
CS	Calcium silicate
XG	Xyloglucan
CNF	Cellulose nanofibrils
PEC	Poly electrolyte complex
DOX	Doxorubicin
OSA	Octenyl succinic anhydride
A549	Epithelial cancer cell line
AgNPs	Silver nanoparticles
Nm	Nanometer
CMT	Carboxy methyl tamarind
PLA	Poly lactic acid
PMMA	Poly methyl methacrylate
FTIR	Fourier transformed infrared
HAP	Hydroxy apatite
RFID	Radio frequency identification
<i>C. odorata</i>	<i>Chromolaena odorata</i>
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
TSX	Tamarind seed xyloglucan
<i>S. epidermidis</i>	<i>Staphylococcus epidermidis</i>
HEMA	Hydroxyethylmethacrylate
HaCaT	Human keratinocytes
CMTKG	Carboxy methyl tamarind gum
MSC	Mesenchymal stem cells
SiO ₂	Silicon dioxide
RAW 264.7,	Monocyte/macrophage cell line
HUVEC	Human umbilical vein endothelial cells
SBF	Simulated body fluid
ECM	Extra cellular matrix
FITC	Fluorescein isothiocyanate
MRI	Magnetic resonance imaging
IONPs	Iron oxide nanoparticles
SERS	Surface enhanced raman spectroscopy
TX	Tamarind xyloglucan
TKP	Tamarind kernel polysaccharide
CMTG	Carboxy methyl tamarind gum
ECM	Extra cellular matrix
LoD	Loss on drying
EnE	Encapsulation efficiency

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Chapter 17

Chitosan-derived Biomaterials in Cancer Therapeutics and Biomedical Imaging

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17.1 Introduction to Anti-cancer Drug Delivery Systems

Cancer has reflected a concerning consistency in its mortality statistics, and it has been one of the leading malignant diseases worldwide for years. It is the name given to a collection of related conditions that develop due to uncontrolled cell division. It may generate in any body part and has the potential to spread to other regions.^{1,2} Many anti-cancer drugs have been developed that can inhibit the growth and proliferation of cancer cells. However, in many cases, they show poor clinical efficacy and severe side effects, mainly due to their low bioavailability and poor selectivity. So, for safer, effective, and selective delivery, a drug needs a well-designed drug delivery system (DDS), thus enhancing its bioavailability, target specificity, and cellular uptake.^{3,4} Designing

an effective DDS involves carefully investigating crucial factors such as the drug's category, selecting an appropriate carrier, designing the drug release mechanism, kinetics, and pathway.

A carrier is responsible for safely conveying the drug into the site of interest by protecting against enzymatic degradation. The physicochemical properties of a carrier predominantly control the drug release pattern into systemic circulation and enhance the drug passage through the cellular membrane.⁵ A drug may be bound to the carrier through covalent attachment,^{6–8} encapsulation,⁹ and adsorption.¹⁰ Some examples of essential drug carriers are liposomes, micelles, polymeric particles, peptides, dendrimers, nanoparticles, microparticles, hydrogels, *etc.*

17.1.1 Biopolymeric Carriers for Anti-cancer Agents

Polymers have played a fundamental part in the progression of cancer drug delivery technology. An added advantage has been observed in using biopolymers as they are highly biocompatible, non-toxic, and biodegradable.¹¹ Hence, after completing the dosage regime, no further surgery is needed to remove the remaining polymer as it degrades inside the body and gets cleared.

A broad class of materials such as nanoparticles, microparticles, hydrogels, implantable scaffolds, nanofibers, *etc.*, have been fabricated using biopolymers for the controlled and targeted release of anti-cancer agents. Various stimuli-responsive polymeric systems have been explored for controlled drug release that can respond to specific external physical or chemical stimuli.

Some of the examples of biopolymers used in anti-cancer drug administration are chitosan, silk-fibroin (SF), gelatin, polylactic acid (PLA), poly(lactic acid-co-glycolic acid) (PLGA), hyaluronic acid (HA), poly-L-lysine (PL), polymalic acid, polyglutamic acid (PGA), poly(aspartame), and many more. However, the scope of this chapter covers only the synthesis and properties of various materials fabricated using chitosan as the parent polymer.

17.1.2 Chitosan as a Versatile Biopolymer and Drug Delivery Vehicle

Chitosan is a natural polysaccharide synthesized by the enzymatic or chemical deacetylation of chitin. Chitin is considered the second most abundant polymer after cellulose and can be obtained from various natural sources such as arthropods and insects (exoskeletons), fishes (soft tissues), fungi (cell wall),

mollusks (radulae), and cephalopods (internal shells and beaks), *etc.*^{4,12} In the deacetylation process, the acetyl group of chitin is replaced by a reactive amino ($-\text{NH}_2$) group, which makes chitosan possess two reactive species, *i.e.*, hydroxyl and amino, thereby improving its solubility, degradability, and ability to form a wide range of derivatives.^{13,14} As it is a weak base, it is insoluble in organic and aqueous mediums. However, it is readily soluble in dilute aqueous acid solutions below its pK_a value of 6.5, as at this pH, the glucosamine units get converted to soluble $\text{R}-\text{NH}_3^+$ form. It is mostly solubilized in 1% (v/v) aqueous acids such as acetic acid, formic acid, hydrochloric acid, *etc.*, for various applications.^{15,16}

Chitosan finds wide application in drug delivery and biomedical applications owing to its exclusive biological properties. It is highly biocompatible, non-toxic, biodegradable, mucoadhesive, hemostatic, and antimicrobial, and binds to mammalian and microbial cells.¹⁷ Depending upon the physiological pH condition, the development of a positive charge in chitosan enables it to undergo electrostatic or hydrogen bonding interaction with the mucous membrane, which possesses a negative charge owing to the existence of sialic acid and sulfate residues.¹⁸ Sialic acid is also distributed throughout the tissue, including in the serum, saliva, breast milk, urine, and cerebrospinal cord.¹⁹ Chitosan can be easily formulated into conjugates, nanoparticles, microparticles, hydrogels, films, nanofibers, scaffolds, and cross-linked compounds, which can be used as drug delivery cargo. It also acts as a reducing agent for the synthesis of various metal nanoparticles. Thus, a wide range of chitosan-derived biomaterials has been synthesized for anti-cancer agents that are discussed in the upcoming sections.

17.2 Chitosan-based Particulate Systems

Chitosan-based particulate systems have shown promising efficacy in intravenous, oral, and topical anti-cancer drug administration.^{20–24} The size and morphology of the particles play a vital role in drug transportation across the biological membranes. Different techniques have been adopted in the formulation of the chitosan particulate system, and the choice of the process is dependent on various parameters such as size and stability of the particles, reproducibility of the kinetics, and residual toxicity of the end products.²⁵

17.2.1 Method of Preparation of Chitosan Nanoparticles and Microparticles for Anti-cancer Agents

The synthesis of chitosan nanoparticles was first reported by Ohya *et al.* in 1994 for the administration of a chemotherapeutic drug, 5-fluorouracil (5-FU), using an emulsion and glutaraldehyde (GA) cross-linking technique.²⁶ Since then, several methods have been proposed for its synthesis, which includes ionotropic gelation, electrospraying, emulsification, reverse micellar process, *etc.*^{27–30} Among these, ionotropic gelation is the most investigated as it involves simple and mild reaction conditions. It involves spontaneous self-assembly of the polymeric chain to form particulates by ionic interaction between the oppositely charged cationic chitosan and polyanions.³¹ Sodium tripolyphosphate (TPP) is a multivalent anion usually used in the process as it is non-toxic, involves mild reaction conditions (without any chemical crosslinkers and high temperature), facilitates gel formation, and is suitable for scaling up.³² The interaction between the ions is highly dependent on the solution's pH, which affects the charge density of chitosan and TPP. Calvo *et al.* first proposed the protocol for synthesizing chitosan nanoparticles using TPP to deliver protein molecules, where low molecular weight chitosan was solubilized in 0.05% acetic acid (v/v), and the pH was adjusted to 5.5 using sodium hydroxide solution. TPP solution with a final concentration of 0.25 mg ml^{-1} was dropwise added to the chitosan solution by varying the chitosan : TPP ratios under continuous stirring at room temperature.³³

Researchers have investigated various parameters that might affect the particles' properties in the ionotropic gelation technique. It was inferred that the size of the particles was highly influenced by the chitosan concentration, molecular weight, degree of deacetylation, chitosan to TPP ratio, and pH of the medium. Increasing the chitosan concentration increases the particle size, which eventually leads to larger aggregates above a critical concentration, which depends on the molecular weight of chitosan and the pH of the medium.³⁴

The anti-cancer drugs are bound to the chitosan particulate primarily by electrostatic interaction, hydrogen bonding, hydrophobic interaction, and covalent conjugation. For embedding the drug into the polymeric matrix, the drug is added to the particulate system during the preparation steps (incorporation). While for adsorption on the particulate surface, the drugs are added after the formulation (incubation). The release of drugs from the particulates is governed by various mechanisms such as swelling of the chitosan matrix, diffusion of the adsorbed or entrapped drug through the matrix, and

erosion or degradation of the polymer. In some cases, an initial burst release of the drugs can be seen, which is primarily due to the generation of pores in the matrix due to swelling and diffusion of the adsorbed drugs on the particulate surface.^{35,36}

17.2.2 Significance of Chitosan Nanoparticles in Anti-cancer Drug Delivery

To increase the bioavailability of an anti-cancer drug, it has to be specifically directed into the site of interest with minimum loss. Hence, a drug has to be attached to a suitable carrier responsible for its safe and effective administration. Due to the effects of size-dependent enhanced permeability and retention (EPR), the chitosan nanoparticles render prolonged circulation time and improved tumor-site accumulation.³² Moreover, the mucoadhesive nature of the particles facilitates increased drug adsorption and bioavailability because of the increased drug-mucosal layer contact time. The adherence of the particles to the mucosal lining also enables prolonged site-specific drug release, which decreases the dosage frequency.³⁷

Chitosan nanoparticles provide provision for the encapsulation of both hydrophilic and hydrophobic drugs. The water-soluble drugs are dissolved in the chitosan solution before the nanoparticle formation. *E.g.*, in a report, the doxorubicin hydrochloride (DOX-HCl) is added to the chitosan solution before the addition of TPP for nanoparticle formation.³⁴ Hydrophobic drugs face a high risk of failure as their solubility factor influences their encapsulation, pharmacokinetics, and pharmacodynamics.³⁸ As a result, the delivery of hydrophobic anti-cancer drugs can show poor absorption and bioavailability. Chitosan nanoparticles can minimize this problem by enhancing the aqueous drug solubility, bioavailability, and systemic absorption.³⁹ They can be custom-made to encapsulate the hydrophobic anti-cancer drugs, which is highly desirable, as several of the best-known anti-cancer agents such as doxorubicin (DOX), paclitaxel (PTX), camptothecin (CPT), curcumin (CUR), *etc.* are hydrophobic. *E.g.*, Zu *et al.* synthesized methoxy poly(ethylene glycol) (PEG) grafted nanoparticles of carboxymethyl chitosan, which showed increased cellular uptake of DOX and effectively inhibited tumor cell proliferation.⁴⁰ PTX loaded chitosan nanoparticles were demonstrated by Li *et al.*, which showed a high drug loading efficiency of $94.0 \pm 16.73\%$ with sustained release, high cellular uptake, and increased cytotoxicity.⁴¹ Trickler *et al.* synthesized chitosan and glyceryl monooleate nanoparticles with a hydrophobic inner-core and

hydrophilic coating by a multiple emulsion (o/w/o) solvent evaporation technique. The nanoparticles exhibited sustained release of PTX with minimized side-effects due to enhancement in cellular uptake by 4-fold and reduction in IC_{50} by 1000-fold.⁴² Similarly, Min *et al.* synthesized a water-soluble carrier for camptothecin by conjugating the 5 β -cholanic acid group (hydrophobic) to the glycol chitosan (hydrophilic). The nanoparticles exhibited a high drug loading efficiency of 80% with significant anti-tumor effects and high tumor-targeting ability.⁴³

Stimuli-responsive chitosan nanoparticles have been explored for controlled and on-demand drug administration. One of the essential factors in the tumor environment is its high local acidic condition (pH range of 5–6) compared with normal tissues, which occur due to the metabolization of glucose to lactic acid by cancer cells. The pH-responsive behavior of the nanoparticle system can be employed for localized drug release. Vivek *et al.* formulated chitosan nanoparticles by a solvent evaporation and emulsification process for the pH-responsive release of tamoxifen. The release of the drug occurred more rapidly in pH 4.0 and 6.0 than 7.4.⁴⁴ Similarly, Unsoy *et al.* synthesized magnetic chitosan nanoparticles for pH-responsive delivery of DOX. With a decrease in pH, there was an increase in the chitosan swelling ratio, hence the drug release. The nanoparticles exhibited maximum drug release at pH 4.2 and were relatively stable at pH 7.4.⁴⁵

Photoresponsive chitosan nanoparticle systems have also been designed where the release of the drug is controlled by light of particular wavelengths. Such a system has been reported where chitosan is conjugated to the drug *via* a photo-linker of the *o*-nitrobenzyl group, which was further formulated into nanoparticles. The nanocarrier showed uniform cleavage of the linker upon irradiation with a light of wavelength 365 nm. This was analyzed using a UV-visible spectroscopic technique, which exhibited a rise in the characteristic peak intensity (300–325 nm) of the *o*-nitrosobenzaldehyde that is generated on the cleavage of the photo-linker (see [Figure 17.1](#)).⁷

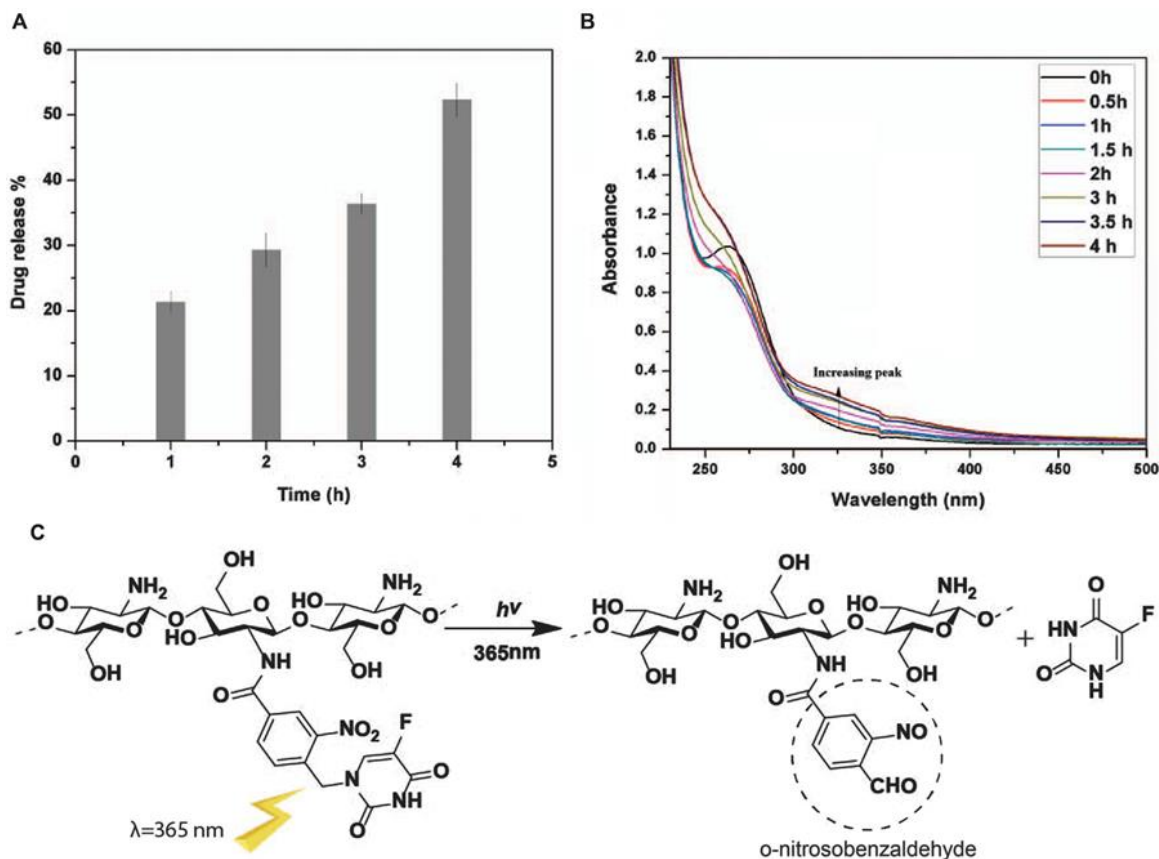


Figure 17.1 (A) Drug release kinetics from photoresponsive nanoparticles; (B) UV-visible spectra, showing the generation of *o*-nitrosobenzaldehyde peak upon irradiation; (C) drug release mechanism from the nanoparticle. Adapted from ref. 7 with permission from Elsevier, Copyright 2019.

Various cancer cell targeting groups such as folic acid (FA), biotin, aptamer, and monoclonal antibodies have been attached to chitosan nanoparticles for target-directed drug delivery of anti-cancer drugs which have been discussed in the chitosan conjugate section.

17.2.3 Significance of Chitosan Microparticles in Anti-cancer Drug Delivery

Microparticles/beads are spherical, micron-, or nano-sized polymeric entities primarily employed in oral and depot release of drugs and can be tuned for controlled and sustained release.^{46–48} The chitosan hydrogel beads consist of cross-linked chitosan chains with tangled mesh structures, providing drug entrapment and swell in physiological conditions to release the drug. In the

depot drug delivery system, particles bigger than 5 μm are generally used for the adsorption or encapsulation of drugs.⁴⁹ The drug release from the microparticles can be controlled by physically cross-linking the particles using TPP and avoiding the usage of toxic reagents. It has been observed that the release kinetics is highly affected by the TPP concentration and gelation time.⁵⁰ Lin *et al.* synthesized TPP cross-linked 5-FU loaded chitosan PEG microparticles and analyzed the effect of various parameters on the drug release behavior. The drug release was retarded by the following factors.⁵⁰

(a) Increasing chitosan concentration which increases the viscosity and leads to greater ionization of the amine group.

(b) pH of the releasing medium, which affects the microparticle structure.

(c) TPP concentration, which increases the cross-linking density, thereby increasing the compactness of the network, and delays diffusion.

Increasing the cross-linking time, which increases the drug–microparticle interaction.

Table 17.1 demonstrates the use of chitosan nanoparticles and microparticles in delivering various types of anti-cancer agents.

Table 17.1 Chitosan particulates for the delivery of anti-cancer drugs.

Chitosan particulates	Methods	Anti-cancer drug	Ref.
Nanoparticles	Ionotropic gelation using TPP	5-Fluorouracil	51
	Ionotropic gelation using TPP	5-Fluorouracil	52
	Ionotropic gelation using TPP	5-Fluorouracil	53
	Solvent evaporation	Tamoxifen	44
	Ionic interaction	Doxorubicin	34
	Ionotropic gelation using TPP	Doxorubicin	54
Microparticles	Emulsion	5-Fluorouracil	55
	Emulsion and ionotropic gelation	5-Fluorouracil	56
	Phase inversion	5-Fluorouracil	50
	Ionotropic gelation	Capecitabine	57
	Ionotropic gelation	Curcumin	58

17.3 Chitosan-derived Metal Nanoparticles

Noble metal nanoparticles have been a versatile agent that finds wide attention in the biomedical sector due to their extraordinary properties, relying on their shapes, size, and chemical compositions. The size of the nanoparticles is a crucial factor that governs their interaction and behavior toward cell components. High surface-area-to-volume ratio, wide-ranging optical properties, ease in formulation, and functionalization make them effective candidates for cancer treatment.^{60,61} Some of the techniques used in the synthesis of these particles are chemical reduction, electrochemical, photochemical, sonochemical, and biosynthesis approaches. Among these, the biosynthetic approach has been of greater choice than the other techniques due to environmental and toxicity issues related to the other techniques.^{62–64}

In the formulation of metal nanoparticles, chitosan helps both in its formation and functionalization. Chitosan possesses many hydroxyl groups that can form complexes with metal ions, thus allowing appropriate control over the shapes and sizes of the formed nanoparticles and minimizing their cytotoxicity.^{65,66} Upon adding chitosan to the reaction mixture, it can interact with the nanoparticles with electrostatic interaction if the nanoparticles are negatively charged; otherwise, they get adsorbed on the nanoparticle surface and form a shell around it.⁶⁷ Functionalizing the surface of the nanoparticles with chitosan can impart various properties such as mucoadhesiveness, increased tissue penetration, cell interaction, antibacterial effects, and physicochemical stability.⁶⁸

Various types of nanoparticles, such as gold, silver, copper oxide, titanium oxide, zinc oxide, and iron oxide, *etc.*, have been synthesized using chitosan for cancer therapeutics.^{69–71} Gold nanoparticles synthesized using chitosan as the reducing and stabilizing agents have been used to deliver PTX and DOX.^{72–74} Similarly, silver nanoparticles synthesized using chitosan have been used to deliver 5-FU, 5-fluorocytosine (5-FC), and DOX.^{10,75,76}

17.3.1 Synthesis Protocol of Gold and Silver Nanoparticles Using Chitosan

The synthesis of gold nanoparticles using chitosan follows a simple one-step protocol. Briefly, chitosan solution in 1% aqueous acetic acid solution is filtered

using a 0.45-micron syringe filter. Gold chloride (HAuCl_4) solution is added in a dropwise manner to the chitosan solution kept under continuous stirring. The temperature is maintained at 100 °C. The formation of the nanoparticle is indicated by a color change of the solution to reddish pink (see [Figure 17.2B](#)). The formation of the nanoparticles can be determined by UV-Vis spectroscopic scan, which exhibits an absorption maximum at a wavelength of 518–520 nm due to surface plasmon resonance (SPR) (see [Figure 17.2A](#)).⁷⁷ The size of the nanoparticles can be analyzed by using transmission electron microscopy (TEM)/field emission transmission electron microscopy (FETEM) (see [Figure 17.2D](#)), and the size distribution histogram can be calculated using ImageJ software (see [Figure 17.2C](#)). Similarly, silver nanoparticles can also be synthesized by a one-step protocol in which silver nitrate solution (AgNO_3) is added dropwise to the chitosan solution, and heated at 90 °C. The nanoparticle formation is indicated by the color change of the reaction mixture to light yellow and finally to brown. Like gold nanoparticles, the silver nanoparticles' formation can also be evaluated by UV-Vis spectroscopy, which shows an absorption maximum between 400–430 nm due to SPR.

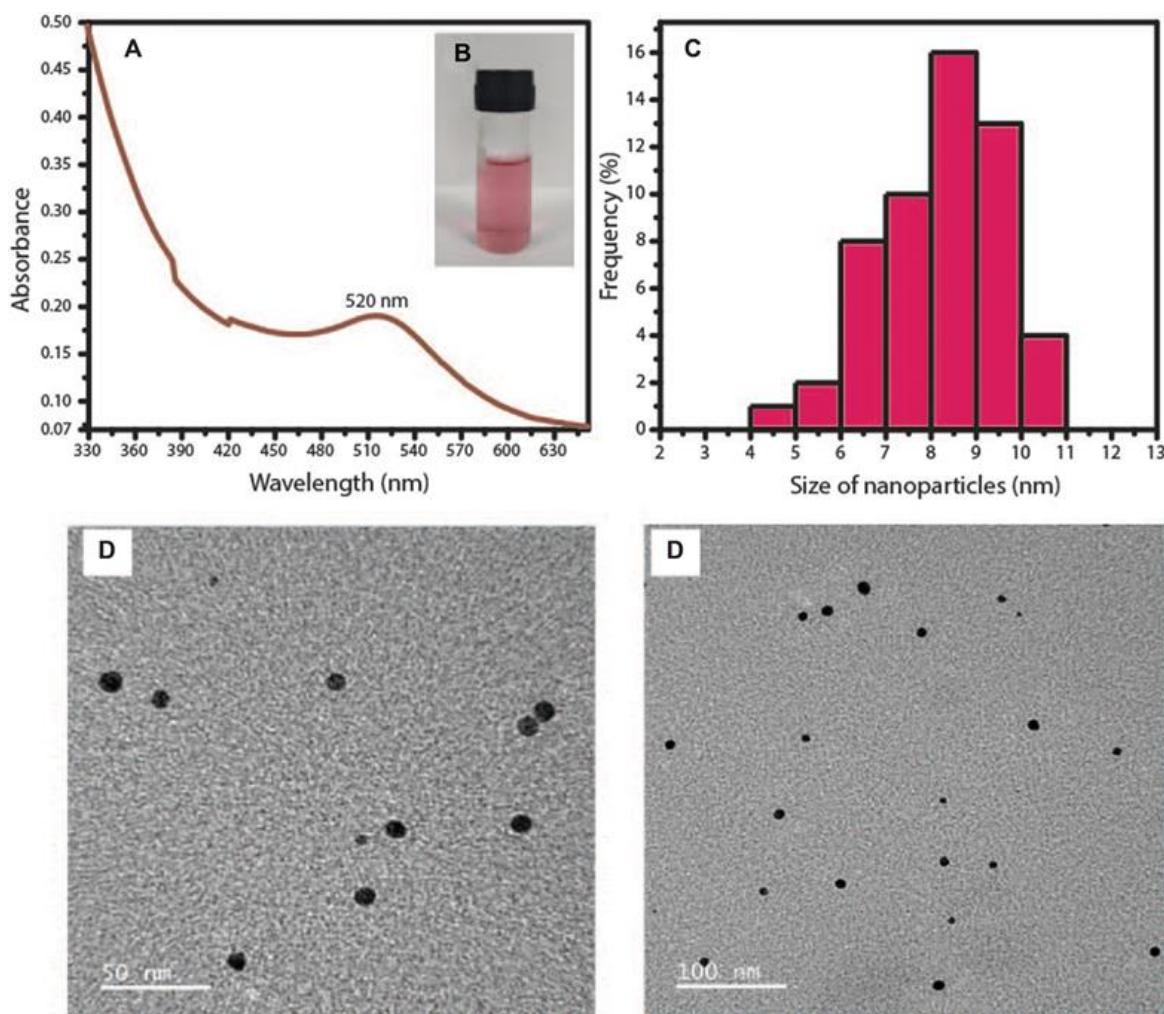


Figure 17.2 UV-Vis absorption spectra. (B) image display in reddish-pink color; (C) size distribution histogram; (D) FETEM micrograph of gold nanoparticles. Adapted from ref. 77 with permission from Elsevier, Copyright 2021.

17.3.2 Significance of Chitosan-derived Metal Nanoparticles in Cancer Therapeutics

Chitosan reduced metal nanoparticles possess the properties of the nanoparticles along with the functional groups and pH-sensitive behavior of chitosan that can be utilized for the effective delivery of anti-cancer agents. Manivasagan *et al.* have reported the synthesis of gold nanoparticles using chitosan oligosaccharides to deliver PTX and photoacoustic imaging of MDA-MB-231 cells. This synthesis procedure was simple and was found to be suitable for large-scale formulation, and is less time-consuming, more cost-effective, environmentally friendly, and safer for clinical research. The nanoparticles

cytosine deaminase (CD). This minimizes the off-target effects as this enzyme is absent in mammalian cells and is only expressed in certain types of bacteria and fungi. For comparative purposes, blank silver nanoparticles and 5-FU loaded silver nanoparticles were also synthesized. The CD activity study showed that the enzyme was particular and effective toward the 5-FC loaded silver nanoparticles.¹⁰ Thus, the system can be used as a prodrug for the targeted and controlled release of 5-FU.

17.4 Chitosan-based Nanocomposites

Biopolymeric nanocomposites are multiphase materials that are made of nanomaterials embedded in a biopolymeric matrix. They consist of two or more phases with different compositions where at least one phase is in the nanometer range. The nanocomposite formation has attracted huge attention to the material and biomedical field as this technique not only enhances the properties of the existing polymer but also incorporates new properties to it. Chitosan-based nanocomposites have been effective as anti-cancer drug delivery carriers as the incorporation of nanoparticles can enhance various properties depending upon the type of the nanomaterial, such as an increase in drug encapsulation efficiency, stimuli responsiveness, and cytotoxicity, *etc.* For example, Arias *et al.* synthesized gemcitabine entrapped Fe_3O_4 /chitosan nanocomposites with multifunctional properties. The presence of Fe_3O_4 incorporated magnetic properties to the material, which resulted in its responsiveness to the magnetic gradient. The nanocomposite exhibited *in vitro* heating behavior upon exposure to a high frequency oscillating electromagnetic gradient. They can produce heat up to 45 °C, which can damage the tumor cells. The nanocomposite also displayed chitosan-induced pH responsiveness, greater drug loading efficiency, and a retarded drug-releasing profile.⁸⁰ Nivethaa *et al.* formulated chitosan/gold nanocomposites for the delivery of 5-FU. The gold nanoparticle was synthesized by the chemical reduction method using sodium borohydride, and the chitosan nanoparticle was prepared by using the ionic gelation technique using TPP. The nanocomposite was toxic toward carcinogenic cell lines and non-toxic toward normal cell lines.⁵³ Nivethaa *et al.* also synthesized chitosan/silver/carboxylated multiwalled carbon nanotubes (MWCNT) nanocomposites. The presence of MWCNT induces a sustained and prolonged release profile of the drugs. It also enhanced the cytotoxicity of the material toward MCF-7 cell lines. Thus, the incorporation of MWCNT enhanced the release profile and cytotoxicity.⁸¹

Magnetic montmorillonite (mMMt)-incorporated κ -carrageenan/chitosan hydrogels were reported by Jafari *et al.* for pH-responsive and magnetic field responsive delivery of sunitinib. The incorporation of mMMt significantly increased the drug loading efficiency from 69% to 96%. There was higher and sustained drug release at an acidic pH of 5.5 than at a pH of 7.4. The release of the drug was faster in the presence of an external magnetic field.⁸² Graphene oxide (GO) nanocomposites with chitosan and dextran were developed by Xie *et al.* using a non-covalent layer-by-layer assembly technique (see Figure 17.4). DOX was attached to the nanocomposite *via* π - π stacking and electrostatic interaction.

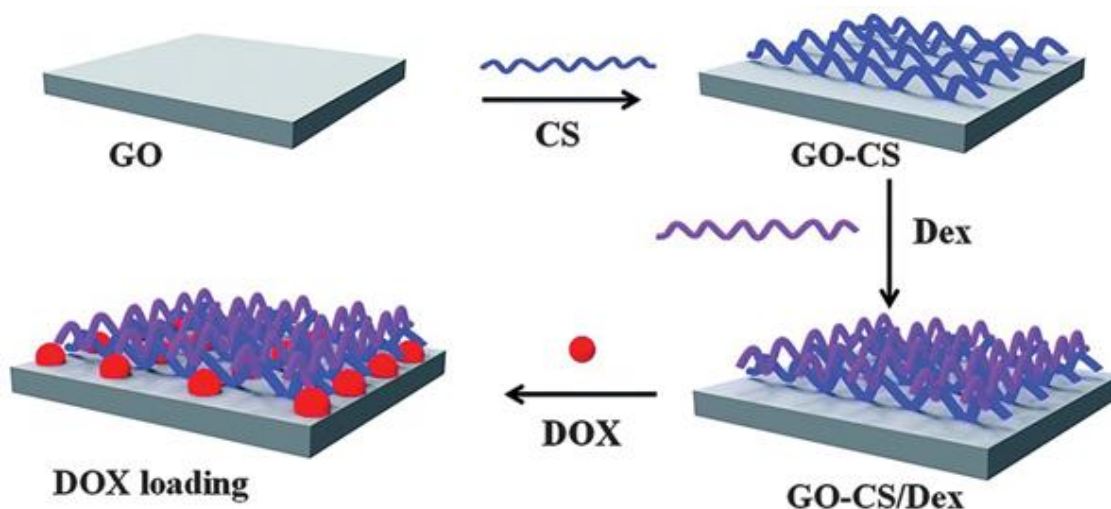


Figure 17.4 Schematic representation of the synthesis of a graphene oxide–chitosan/dextran (GO–CS/Dex) nanocomposite with DOX loading. Adapted from ref. 83 with permission from the Royal Society of Chemistry.

Upon modification by chitosan/dextran, the dispersibility and stability of the GO under physiological conditions has been significantly enhanced compared to the unmodified one. It has also increased the specificity of protein binding and pH-responsive behavior with higher release in low pH. The pure nanocomposites were inert toward MCF-7 cells but showed potent cytotoxicity upon DOX loading.⁸³ Amini-Fazl *et al.* synthesized 5-FU loaded chitosan/polyacrylic acid/ Fe_3O_4 magnetic nanocomposite hydrogels. The Fe_3O_4 nanoparticle is stabilized by chitosan, which gets adsorbed on the nanoparticle by electrostatic attraction. The acrylic acid is copolymerized on the surface of chitosan for the formulation of the nanocomposite. The drug loading efficiency

was increased in the presence of Fe_3O_4 . The drug release study unveiled the enhancement of drug dosing stability for an extended period in the colon and rectal conditions.⁸⁴

17.5 Chitosan-based Hydrogels

The discovery of better scaffolds, matrices, and drug delivery systems is becoming extremely important as health care costs rise and the population grows. The use of multifunctional scaffolds (particularly smart hydrogels) instead of non-degradable scaffolds helps minimize disease risk.

Hydrogels are a three-dimensional hydrophilic polymeric network formed by cross-linking of natural and synthetic polymers. Due to the chemical and physical modifications of the linear chains of the polymer, a hydrogel can retain a large amount of water while maintaining its structure. For a material to be a hydrogel, the water weight of the material must be at least 10 percent of the total weight volume. The network's hydrophilicity gets enhanced by the existence of hydrophilic moieties such as $-\text{CONH}_2$, $-\text{OH}$, $-\text{NH}_2$, $-\text{COOH}$, $-\text{SO}_3\text{H}$, and $-\text{CONH}$. The increased hydrogel expansion and occupation of large volumes caused by this physicochemical mechanism results in swelling behavior.⁸⁵ It's a kind of material with extraordinary properties that make it ideal for biological applications. Hydrogels are a class of materials with unique physical characteristics, making them appropriate for a wide range of biomedical applications.^{86,87} Hydrogels have been used in cancer treatment and tissue engineering due to their exceptional, unique porous structure, high mechanical properties, swelling properties, biocompatibility, and structural resemblance to the extracellular matrix in its natural state.⁸⁸ Hydrogels that have been specifically intended to respond to external stimuli such as temperature, pH, magnetic field, electric field, ultraviolet light, and ionic strength as well as to internal stimuli such as chemical architecture, initiators, and initiator's conditions, can be a deserving candidates for biomaterial applications.⁸⁵ Hydrogels can be formulated from synthetic or natural polymers such as chitosan, HA, PEG, sodium alginate, PVA, and cellulose.^{87,89,90} Chitosan, in particular, is a naturally occurring amino polysaccharide with widespread availability that does not cause acute cytotoxicity.⁹¹

Tiny molecular drugs, macromolecular therapeutics, and even cells could be encapsulated and released using injectable hydrogels. With the rapid advancement of cancer immunotherapy, injectable hydrogels have received

much attention for local immunomodulation to improve systemic anti-cancer immune responses, allowing for more successful immunotherapy at lower doses.

17.5.1 Classification of Hydrogels in Current Biomedical Applications

Hydrogels can be classified in accordance with the current biomedical applications as follows (see [Table 17.2](#)).

Table 17.2 Classification of hydrogels.

Classification of hydrogels					
Source	Crosslinking	Preparation methods	Ionic charge	Biodegradability	Size
Natural	Physical cross-linking	Homopolymeric	Anionic	Biodegradable	Nanogel
Synthetic	Chemical crosslinking	Copolymeric	Cationic	Non-biodegradable	Microgel
Hybrid		Interpenetrating	Nonionic		Macrogel

17.5.2 Method of Preparation of Chitosan Hydrogels for Anti-cancer Therapeutics

Chitosan hydrogels can be produced in a wide range of geometries, shapes, and it can be prepared from different compositions, including microparticles, microspheres, powders, films, capsules, beads, sponges, textile fibers, and inorganic composites.⁹² The hydrophilic polymer, which is the main structural component of a hydrogel, is influenced by a variety of factors such as the quantity of water required to absorb it and the technique of linking the polymeric chains within the network.⁴ Several approaches have been proposed for its synthesis: chemical method, physical methods, ionic complexes, polyelectrolyte complexes, physical mixtures, secondary bonding, thermo-reversible hydrogel, and hydrophobic interactions cross-linking by ultraviolet irradiation, cross-linking by high temperature, and cross-linking by high pH. The amino and hydroxyl groups of chitosan can interact with the other cross-linking agents to form stable chemical hydrogels.^{93,94} Chemical hydrogels, also called covalently cross-linked chitosan hydrogels, are formed by permanent

covalent connections between amino and hydroxyl groups of chitosan and the various polar groups on the crosslinkers that are stable enough to withstand the harsh acidic environment of our stomach to achieve the sustained release of the required drug molecule. This is the most studied and used method to create permanent cross-linking between chitosan polymers. Wichterle *et al.* developed the first chemically cross-linked hydrogel in 1960 by polymerizing 2-hydroxyethyl methacrylate (HEMA) with ethylene glycol methacrylate as a cross-linking agent.⁹⁵ The most commonly used chemical cross-linking agents for the preparation of hydrogels are aldehydes, epoxy compounds, and esters.^{96,97} The aldehyde group of GA and the amino group of chitosan result in the formation of a covalent imine bond *via* Schiff's base reaction.⁹⁸ In biomedical applications, GA cross-linked chitosan and PVA hydrogels were used.⁹⁹ Crosslinking of chitosan and GA has many advantages over the other cross-linking agents. It has mild experimental conditions, it is easy to synthesize, and has satisfactory biocompatibility of the reactants. However, the downstream processing is very challenging for neurotoxic GA.¹⁰⁰

Chitosan hydrogels with genipin cross-linking have also been prepared.^{101–103} Genipin is of natural origin and is highly proficient in attaching biological tissues and biopolymers by covalent cross-linking. It is less toxic and more stable than other crosslinkers such as GA and is highly effective in cross-linking polymers with amine groups, such as chitosan.^{102,104,105} Various reversible interactions combine to form physical hydrogels. Ionic interactions, as seen in ionically cross-linked hydrogels and polyelectrolyte complexes (PEC), or secondary interactions as in chitosan/PVA complexed hydrogels, grafted chitosan hydrogels, and entangled hydrogels, are some examples.⁹⁸ Other non-covalent interactions such as hydrophobic, electrostatic, and hydrogen bonding can also generate the physically cross-linked chitosan hydrogel. It can be made by simply combining the ingredients under suitable reaction conditions. The life span of these hydrogels is short, ranging from several days to a month. Owing to the lack of hazardous chemical crosslinkers, they are ideal for short-term drug release applications. However, due to the relatively low mechanical strength, they are only useful in a few situations.

There are three main basic loading techniques between drug and hydrogel systems: encapsulation, penetration, and covalent bonding.^{4,106} Most anti-cancer agents use a stimuli-responsive or smart chitosan hydrogel system, which can release the anti-cancer agents in response to external stimuli such as temperature, pH, and light. The open pore-like topographies of these smart

chitosan hydrogels efficiently promote drug release in response to various specific stimuli.^{107–109} To increase the drug loading efficacy, drugs can be entrapped within the polymeric matrix through covalent bonding or a physical method during the material gelation.¹¹⁰

5-FU, polyoxometalates, DOX, and CUR are some anti-cancer drugs that can be carried and delivered by chitosan hydrogels using GA and TPP as chemical cross-linking agents.¹¹¹ Since each approach has its own set of benefits and drawbacks, the characteristics of the drug and the hydrogel property needs to be taken into consideration.

17.5.3 Significance of Chitosan Hydrogels in Anti-cancer Therapeutics

Chitosan is a natural cationic polymer that can adhere very firmly to the negatively-charged cell surface. Chitosan-based matrices have successfully delivered small or large molecular weight anti-cancer agents in a controlled manner. Furthermore, the biochemical properties of the chitosan make it a stable therapeutic agent against all the enzymatic and chemical degradation, which excels in drug delivery through oral, transdermal, ocular, and subcutaneous delivery. In addition to its beneficial properties, such as wound healing speed and antibacterial activity, chitosan has also been shown to inhibit tumor cell development.¹¹² It also inhibits tumor-induced metastasis and tumor angiogenesis.^{113,114} In metastatic breast cancer models, intra-tumoral injection of chitosan compounds alone has been proven to have anti-tumor effects. In Balb/c mice, chitosan was identified to stimulate the transformation of macrophages into cytotoxic macrophages and decrease Meth-A tumor development.¹¹⁵ Obara *et al.* also reported that administering PTX-incorporated Az-CH-LA hydrogels to tumor tissues experienced remarkable tumor necrosis and strongly inhibited tumor angiogenesis.¹¹⁶ For DOX administration, an *in situ* gelling chitosan-based dipotassium orthophosphate hydrogel was recently introduced.¹¹⁷ When DOX was incorporated into the hydrogel, it prevented the development of osteolysis, osteosarcomas, and lung metastasis in mice. It also reduced the adverse effects of DOX and minimized systemic toxicity as compared to traditional administration by triggering drug release at local sites.⁴ Injectable hydrogel systems of chitosan have many advantages over systemic chemotherapy, including reduced toxicity in normal tissues, localized and sustained drug delivery in the tumor region, more effective cell apoptosis, and

tumor growth inhibition.¹¹⁸ To achieve successful and localized therapy, Chang *et al.* reported a self-healing chitosan/PVA hydrogel. The hydrogel maintained a significant drug concentration near the cancer cells and allowed for pH-controlled drug release at the lesion site.¹¹⁹ In a breast cancer xenograft mouse model, Azab *et al.* examined chitosan hydrogels filled with I-norcholesterol. The tumor development was slowed, and the drug that was released decreased tumor recurrence and metastatic spread by close to 70 percent.¹²⁰

Chitosan-modified hydrogels, in particular, have shown tremendous potential in releasing drugs with a prolonged release profile due to their porous structure, making them useful for the delivery of chemotherapeutic agents like PTX in cancer therapy.¹¹⁶ Zhang *et al.* developed a nanocomposite hydrogel for PTX-loaded chitosan micelles (PTX-M) and gold nanorod (GNR) in photothermal chemotherapy. The thermo-sensitive hydrogel matrix that was created successfully has delivered GNR and PTX-M to the tumor site. The GNR-mediated photothermal damage was caused by laser ablation.¹²¹

The molecular chain of chitosan contains many hydroxyl and amino groups; therefore, it has good adsorption and chelation abilities in the drug delivery system, making it pH-sensitive.¹²² Furthermore, it can be used extensively in anti-tumor drug release due to a pH-gradient near the tumor microenvironment.¹²³ In another investigation, Duan *et al.* used nanogels synthesized from galactosylated chitosan and poly(*N*-isopropylacrylamide) as carriers for oridonin (ORI) against liver tumors.¹²⁴ Zhan *et al.* used 4-arm PEG-benzaldehyde (4-armPEGDA) and *N*-carboxyethyl chitosan (CEC) as a new drug carrier to develop a pH-sensitive injectable hydrogel. Within five days of its administration, the DOX-loaded hydrogel has dramatically reduced the tumor development in hepatocellular carcinoma cell lines.¹²⁵

Grafting tumor-specific moieties on chitosan hydrogels by chemical modification of CS can increase the stability, tumor-targeting ability, and transfection efficiency, respectively. Several active targeting ligands and cell adhesion moieties, such as the arginine–glycine–aspartic acid (RGD) peptide, FA, and lactobionic acid (LA), have been effective when integrated into nanogels to increase tumor targeting and drug accumulation.¹²² Table 17.3 demonstrates the use of chitosan hydrogels in delivering various types of anti-cancer drugs.

Table 17.3 Chitosan hydrogels in anti-cancer drug delivery.

Hydrogel system	Delivering molecule/drug	Cell line	Ref.
CS/HA/GP	DOX	HeLa (cervical cancer)	126
Chitosan/glycerophosphate (CS/GP)	Indocyanine green (ICG)	Hepatocellular carcinoma (HCC)	127
CS and GP	DOX	MCF-7 cells (breast cancer)	128
Chitosan dihydrocaffeic acid (CS-DA) and oxidized pullulan (OP)	DOX and amoxicillin	Hct116 cells (colon tumor cells) and <i>E. coli</i>	129
Chitosan (CS) and polyvinyl alcohol (PVA)	Doxazocin	HeLa cell line (cervical cancer)	130
Gal-CS- <i>g</i> -PNIPAm	Oridonin	HepG2 cells, MCF-7 cells	124
CSSH/Cur-Lip gel	CUR	MCF-7 cells (human breast cancer cell line)	131
Azide-chitosan-lactose (Az-CH-LA)	PTX	3LL cells	116
Chitosan/ β -glycerophosphate (C/GP)	DOX and vaccinia virus-based vaccine	Cervical cancer	132
Chitosan/glyceryl monooleate (C/GMO)	PTX	Mucin producing cancerous cells	133
Chitosan/ β -glycerophosphate (C/GP)	CPT	RIF-1 fibrosarcoma	134

17.6 Chitosan-based Conjugates

In drug delivery, conjugation is generally done for the following reasons:

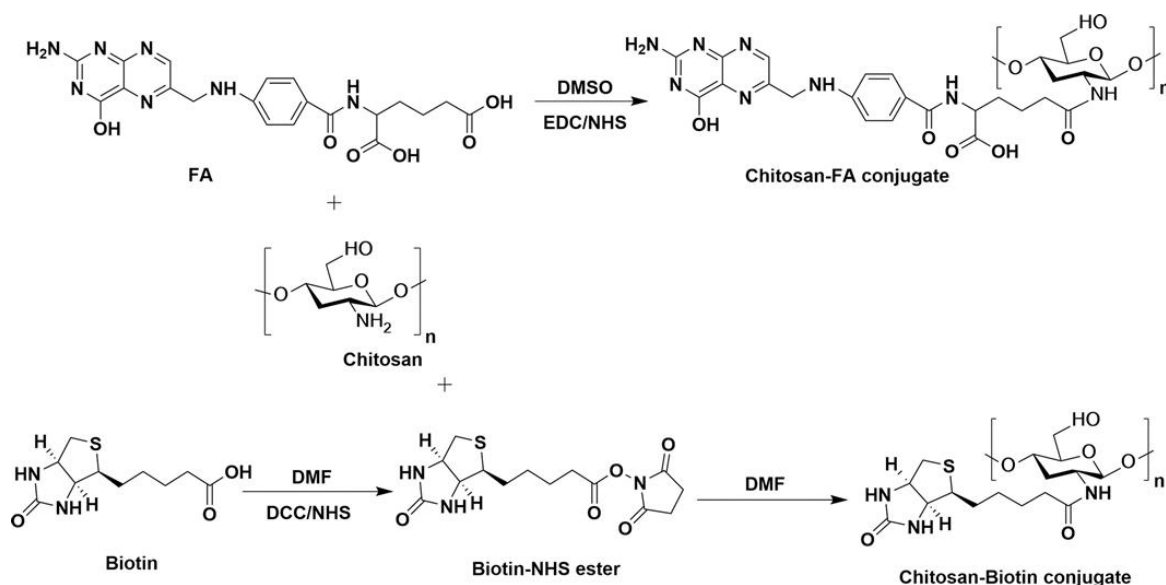
- (a) Imparting some desirable properties such as hydrophilicity, biocompatibility, increased cellular uptake, and cell imaging, *etc.*, to the system.
- (b) Attachment of cell-targeting and stimuli-responsive groups to the system.

(c) Impart stability to the attached drug to avoid premature drug leakage and burst release.

Chitosan conjugates have been a very effective approach in developing anti-cancer drug delivery cargo. The presence of many amino and hydroxyl groups enables the conjugation of various moieties to chitosan *via* amide and ester linkages. This approach has been extensively used to conjugate drug targeting moieties such as folic acid, biotin, glucose, and HA, *etc.* Receptors of these cell-targeting moieties are highly overexpressed on cancer cells; thus, chitosan-targeting moiety conjugates possess the significant properties of chitosan along with a cell-targeting nature. Folate receptor α (FR- α) are glycosyl-phosphatidylinositol-anchored membrane glycoproteins highly present in 90% of ovarian and various epithelial cancer cells. Some cancer cells, such as leukemia, ovarian, and colon cells, *etc.*, express another ligand–receptor type even more than FA, *i.e.*, biotin.¹³⁵ Likewise, glucose moiety interacts with glucose transporters (Gluts) overexpressed in various cancer cells, such as the breast, pancreas, *etc.*, and hyaluronic acid interacts with cluster determinant 44 (CD44) receptors over-expressed on some cancer cell surfaces.¹³⁶ The conjugation of these groups can be done to the free amino group of chitosan by carbodiimide coupling.

As reported by Yang *et al.*, FA-conjugated chitosan has been synthesized that showed high affinity toward colorectal cancer cells. The following protocol conjugated the FA. FA and ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in a molar ratio of 1 : 1 were dissolved in anhydrous DMSO. This solution was slowly added to the chitosan solution in aqueous acetic acid (0.1 M, pH 4.7) and reacted at room temperature for 16 h. The purification was done by using the dialysis method, and the conjugation was analyzed by FTIR spectroscopy.¹³⁷ The conjugation of biotin is generally done by forming a biotin-NHS ester. According to the reported protocol, the ester was synthesized by reacting biotin with dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) in DMF overnight. The by-product, dicyclourea, gets precipitated out of the solution, which is filtered off using filter paper. The compound was then precipitated with the help of diethyl ether. The product was further recrystallized using isopropanol at 70 °C and vacuum dried. Chitosan was conjugated to the ester by dropwise adding chitosan solution in aqueous acetic acid and reacting for 20 h at room temperature. The purification was done

by dialysis.^{138,139} The conjugation protocol of FA and biotin to chitosan is shown in [Scheme 17.1](#).



Scheme 17.1 Synthesis scheme for chitosan–FA conjugates and chitosan–biotin conjugates.

Chen *et al.* synthesized biotinylated chitosan-modified PLGA nanoparticles for the delivery of epirubicin. The MTT assay showed that the conjugated biotin system showed a remarkable increase in cytotoxicity compared to the unmodified ones. The cellular uptake was also increased in the modified material and was evident from flow cytometry and using a confocal microscope.¹³⁹ Similar results have been seen in a work reported by Wu *et al.* (2016), where they have synthesized DOX-loaded biotinylated carboxymethyl chitosan/ CaCO_3 hybrid nanoparticles. The biotinylated materials showed increased cellular uptake and cell inhibition compared to the pure drug and material without biotin.¹⁴⁰ Yang *et al.* conjugated HA and fluorescein isothiocyanate to carboxymethyl chitosan-modified GO. DOX was encapsulated into the material *via* π – π stacking with GO. The release of the drug from the conjugate was highly pH-dependent. There was higher drug release at a lower pH of 5.8 compared to 7.4. There was increased cellular uptake and cytotoxicity with materials containing HA due to receptor-mediated binding.¹³⁶

Apart from targeting moieties, various types of stimuli-responsive linkers can also be attached to the chitosan backbone for the controlled delivery of anti-cancer agents. Lee *et al.* synthesized pH-responsive linker conjugated chitosan

for the delivery of paclitaxel. They attached the drug to chitosan *via* a succinate linker using an EDC/NHS coupling agent. The conjugate exhibited improved hydrophilicity, bioavailability, and cytotoxicity in the mice model.⁸ Lee *et al.* synthesized nanoparticles of HA and glycol chitosan conjugates for simultaneous delivery of DOX and celecoxib. The DOX was attached to the nanoparticles through a pH-responsive moiety and the celecoxib *via* physical loading. The drugs are released from the nanoparticle at a lower pH of 4 and 6, whereas they remain steady at neutral pH. Enhanced efficacy was seen in the case of combined drugs compared to the activity of pristine drugs and nanoparticles.¹⁴¹ Glutathione (GSH) and poly[poly(ethylene glycol)methacrylate] (PMPEG) conjugated chitosan was reported by Li *et al.* The PMPEG was synthesized by reversible addition–fragmentation chain transfer (RAFT) polymerization. The PMPEG possesses brush-like structures and, along with GSH, helps avoid self-aggregation and enhances cellular uptake. The material act as a gene delivery vector which displayed outstanding transfection efficacy in NIH3T3 cells.¹⁴²

17.7 Derivatives of Chitosan as Anticancer Agents

Chitosan is the only naturally-occurring cationic polysaccharide. It has a wide range of applications in biomaterials; despite this, chitosan's use is restricted due to its insoluble nature in neutral or alkaline pH, low absorbability, solid crystalline structure, high viscosity, and high molecular weight. Chemical modification of chitosan can result in many derivatives with improved solubility and a wide range of applications. The amino group and acetamido residue derivatization of chitosan has been shown to give compounds with increased solubility and biological activity.^{143,144} Chemical modifications can not only enhance chitosan's chemical and physical properties, but it also extends its application scope while preserving its remarkable unique properties. Modified chitosan derivatives have improved biodegradability, bioactivity, biocompatibility, and non-toxicity while maintaining the original pharmacological effects of antibacterial, bactericidal, anti-cancer, and antiviral activity.^{145,146} Chitosan, for example, has been grafted with heparin to improve its angiogenic and anticoagulant activity and its affinity for several growth factors.¹⁴⁷

17.7.1 Chemical Modifications of Chitosan

Chitosan's most advantageous feature is its ability to be chemically transformed into a wide range of derivatives. Owing to the existence of a primary alcohol group, a secondary alcohol group, and an amino group, chitosan can be functionalized in the O-position, N-position, and N,O-position.¹⁴⁸ To improve the existing properties of the chitosan, small molecules and ligands can be grafted over the main backbone of the chitosan molecule. The intended application of the final chitosan derivative determines the site of modification. Chitosan and its derivative have wide applications in the field of drug delivery and cancer therapeutics. Thiolated, quaternized, hydrophobic, and chemically-grafted products are some types of derivatization of chitosan that have been found to enhance or add desirable properties to it. The quaternization of chitosan is a well-known approach to increase positive surface charge and superior solubility in physiological pH. Different properties of modified chitosan, like antimicrobial activity and cellular absorption, can be improved by introducing these changes.¹⁴⁹ It generally occurs through three methods – quaternary ammonium substitution, *N*-alkylation, and the epoxy derivative open loop method.

17.7.2 Some Anticancer Derivatives of Chitosan

17.7.2.1 2-Phenylhydrazine (or Hydrazine) Thiosemicarbazone Chitosan

Hydrazine derivatives are synthesized by reacting chitosan with methyl hydrazine-dithiocarboxylate or methyl phenylhydrazine-dithiocarboxylate. The reaction medium used is DMSO at a temperature of 100 °C.¹⁵⁰

17.7.2.2 Carboxymethylchitosan (CMCS)

It is one of the water-soluble derivatives of chitosan. The carboxymethylation occurs primarily at C-6 hydroxyl groups or the NH₂ moiety of chitosan, generating a water-soluble *N,O*-carboxymethyl chitosan compound.¹⁵¹ Yang *et al.* designed a GO-modified carboxymethyl chitosan conjugated with HA as a drug carrier to improve the anti-cancer drug delivery efficiency, specificity, and achieve constructively regulated release. The resulted conjugate was used to encapsulate the anti-cancer drug DOX. A high drug loading capacity of 95% was exhibited. The drug release rate is substantially higher at pH 5.8 than in the neutral condition of pH 7.4. The material can specifically target cancer cells

containing a significant number of CD44 receptors, thus effectively inhibiting their development.¹³⁶ Chi *et al.* developed conjugated carboxymethyl chitosan (CNC) for the delivery of anti-cancer drug norcantharidin, intending to improve its anti-tumor efficacy in clinical use. The conjugates were found to significantly inhibit cell proliferation and induce apoptosis in SGC-7901 cells.¹⁵²

17.7.2.3 *Furanoallocalchicinoid Chitosan*

Colchicine is a 400 Da hydrophobic molecule that penetrates cells passively and binds irreversibly to tubulin, preventing microtubule formation and cell division.¹⁵³ Colchicine is a potent anti-tumor agent because of its highly specific antimitotic activity. High therapeutic doses (50–100 mg m⁻²) cause only minor side effects, the most frequent of which is general gastrointestinal toxicity (5–10%). Furanoallocalchicinoid, a new allocalchicine derivative, was synthesized and conjugated to chitosan.¹⁵⁴ In Wnt-1 breast tumor-bearing mice, the anti-tumor effects of chitosan, furanoallocalchicinoids, and furanoallocalchicine–chitosan conjugate were investigated. Furanoallocalchicine–chitosan conjugates inhibited tumor growth slightly better than furanoallocalchicinoids alone, likely due to better tumor accumulation.¹⁵⁵

17.7.2.4 *Sulfated Chitosan (SCS) and Sulfated Benzaldehyde Chitosan (SBCS)*

Chitosan with an average M_w of 1000 kDa that possesses two –OH groups is selected for synthesizing this derivative.¹⁵⁶ In MCF-7 cells, SCS and SBCS suppressed cell propagation, enabled apoptosis, and blocked FGF-2-induced phosphorylation of ERK. SBCS had a more substantial inhibitory effect and a lower IC₅₀ than SCS. Sulfated and benzaldehyde chitosans appear to be promising candidates for anti-cancer drug development.¹⁵⁷

17.7.2.5 *Chitosan–Thymine Conjugate*

Chitosan–thymine conjugates can be synthesized by reacting chitosan and thymine-1-yl-acetic acid followed by acylation.¹⁵⁸ The pyrimidine nucleoside thymidine's growth-inhibitory and cytotoxic effects on mammalian cells *in vitro* has been recognized for two decades. It has been widely used to synchronize mammalian cells in the S phase (DNA synthesis) of the cell cycle.¹⁵⁹ Silva *et al.* reported that the Ru(II)–thymine complex inhibits tumor cell growth in a

xenograft model and causes DNA damage and apoptotic cell death in HCT116 cells via JNK/p38/ERK1/2-mediated p53-independent signaling.¹⁶⁰

17.7.2.6 Glycol-Chitosan–Mitomycin C Conjugates

Mitomycin is a cytotoxic antibiotic that can be utilized as an anti-cancer drug, but due to its several side effects, its use is restricted. Attachment of a macromolecular prodrug along with an efficient drug carrier can specifically deliver the drug to the tumor site. Through a spacer of the glutaryl group, mitomycin C (MMC) was covalently linked to glycol-chitosan (Gly-chitosan) and *N*-succinyl-chitosan (*N*-Suc-chitosan), and each conjugate was formed as a water-soluble product.¹⁶¹ Jia *et al.* have developed methotrexate (MTX) and MMC-loaded PEGylated chitosan nanoparticles (CS-NPs) as drug delivery systems, with MTX serving as a tumor-targeting ligand. The new drug delivery system has demonstrated a more significant targeting effect in the early stages with the anti-cancer effect in the later stages.

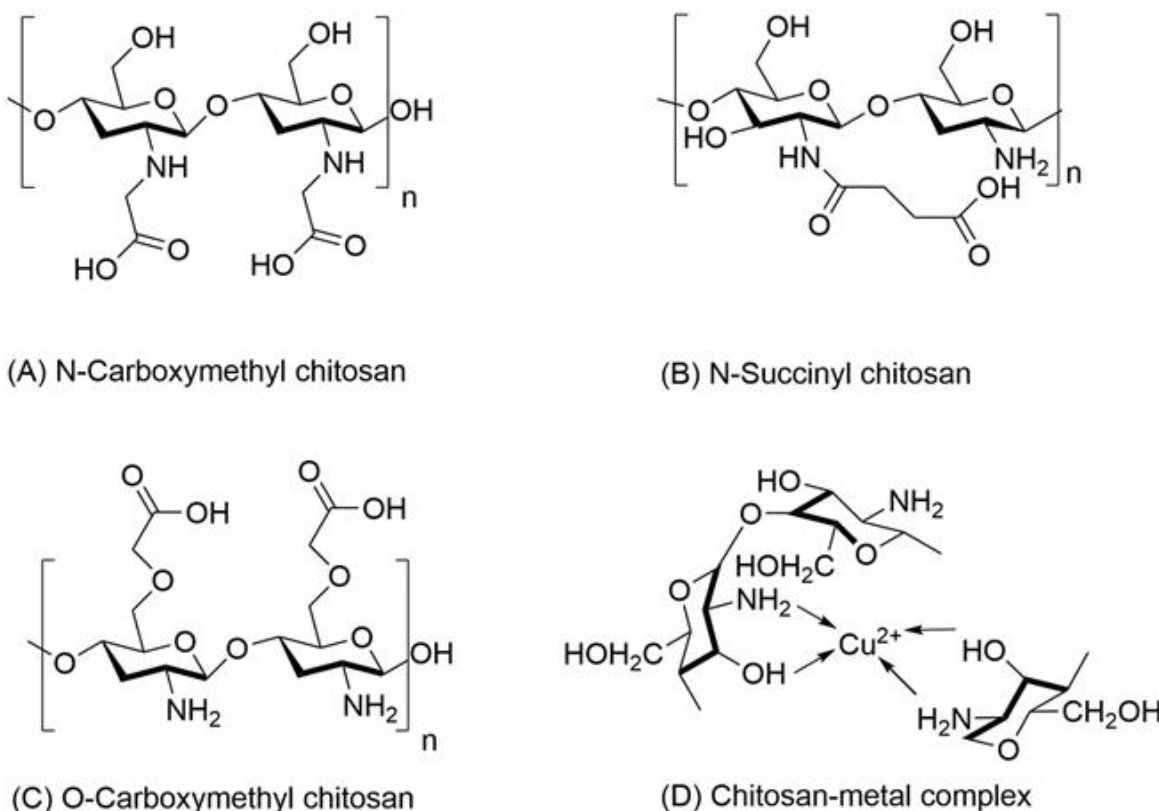
17.7.2.7 *N*-Succinyl-Chitosan

As a drug delivery vehicle, *N*-succinyl CS has many advantages, including increased aqueous solubility and long-term retention in the body.¹⁶² Two units of chitosan are attached to one unit of succinic anhydride by forming amide bonds with both the carbonyl groups of the anhydride molecule via acidic dehydration. The product is water-soluble and thus extracted via precipitating out with the help of a non-polar solvent. The reaction is driven forwards by the formation of a highly stable amide bond.¹⁶³ Luo *et al.* have successfully synthesized an *N*-succinyl-chitosan nanoparticle. NSCNP inhibited the viability of K562 cells with an IC_{50} of 14.26 g ml⁻¹ (24 h); the antiproliferation effect was achieved by inducing synchronous apoptosis and necrosis in K562 cells.¹⁶⁴

17.7.2.8 Chitosan–Metal Complex

Each repeating unit of glucosamine in chitosan has an amine and two hydroxyl groups that can serve as a reactive site with excellent chelation and electrostatic interactions to bind metal ions.¹⁶⁵ Chitosan has been shown to form various metal complexes like chitosan–silver, chitosan–zinc, and chitosan–manganese, and to be active against a variety of bacterial strains, including *Salmonella choleraesuis*, *Staphylococcus aureus*, and *E. coli*.¹⁶⁶ Cab@MPN/CS NPs have a

more extended retention period in tumor tissues and inhibit tumor growth substantially by downregulating Ki67 and CD31 expression.¹⁶⁷ Ai *et al.* prepared copper-loaded chitosan nanoparticles (CuCNPs) for the treatment of osteosarcoma. On cancer cells, CuCNPs had a remarkable anti-cancer effect. CuCNPs are linked to a higher level of mitochondrial ROS production; CuCNPs with a higher ROS generation capacity have increased caspase 3/7 activity.¹⁶⁸ Structures of some common chitosan derivatives are shown in [Scheme 17.2](#).



Scheme 17.2 Some representative structures of common chitosan derivatives.

17.7.3 Significance of Chitosan Derivatives in Anti-cancer Therapeutics

Chitosan and its derivatives are considered as the naturally obtained potential anti-cancer polysaccharide. It can form tight junctions in cell membranes owing to its cationic nature and possession of active functional groups. Various derivatizations are possible with different properties.¹⁶⁹ The ability of $-\text{NH}_2$ and $-\text{OH}$ groups in the C-2, C-3, and C-6 positions of the pyranose ring to extract

the proton from free radicals cause chitosan and its derivatives to have antioxidant properties. Chitosan derivative nanoparticles are highly mucoadhesive, have higher water solubility, and facilitate stronger antigen absorption than chitosan nanoparticles.¹⁷⁰ Chitosan oligosaccharide prevented cell proliferation in the A549 lung cancer cell line by modulating cell autophagy.¹⁷¹ Most scientists have demonstrated that chitosan has low cytotoxicity toward cells, has significantly fewer side effects, and has anticarcinogenic properties. It can inhibit cell growth, cell proliferation, and can induce cell apoptosis.

17.8 Chitosan-based Imaging System for Cancer Diagnosis

Despite the huge technological advancement in disease diagnosis, it is still not at par when it comes to cancer diagnosis. There are only a few principal diagnostic techniques available for cancer, such as X-ray (plain film and computed tomography [CT]), ultrasound (US), magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), positron emission tomography (PET), and optical imaging. Among these techniques, CT, MRI, SPECT, and PET are capable of three-dimensional (3-D) imaging. These are very powerful techniques but still fail to give the desired contrast. The main problem with cancer imaging is that the cell to background ratio is very low and there is non-specific uptake of the contrasting agent by normal tissues.¹⁷² Let us discuss the drawbacks of each of the techniques briefly before discussing the chitosan-based systems.

Despite being the most common and least expensive technique, US has very poor penetrability inside our body, up to approximately 10 cm only inside our body.¹⁷² Compared to US, X-Ray and MRI have better penetrability, but these techniques fail to create an appropriate contrast to the image because of the very low signal to background ratio. X-rays need injectable contrasting agents but are non-targeted and become invisible after only a few-fold dilution in blood.¹⁷² On the other hand, MRI needs a higher magnetic field as high as 7 T to produce good contrast but this much magnetic field results in other problems like increased tissue heating.¹⁷³ The last one is the optical imaging technique, which uses scattering or absorption of light by the cells and provides a good resolution. Optical microscopy is limited to approximately 3 mm depth inside the tissue, which is significantly less to detect cancer in any organ of any living being.

About 10 cm of tissue depth was achieved using tomographic optical spectroscopy and imaging (*i.e.*, diffuse optics). It uses multiple scattering and then reconstructs the image using a computational model to form a 3-D image of thick tissues. Still, with the increasing depth, a significant reduction in resolution is also evident.¹⁷² So, whatever we have discussed so far, it is easily understandable that although we have very powerful tools for imaging, a serious upgrade of technology is still required for cancer cell imaging. So far, the main issue is either the resolution because of the weak signal to background ratio or the limited imaging depth. To address such difficulties, scientists have come up with different techniques involving molecular imaging in combination with existing technologies. Chitosan-based molecular technologies are gaining popularity because of the versatility of chitosan as a biopolymer. This section of this chapter will discuss how chitosan is being used to increase the resolution and sensitivity of cancer cell imaging.

Chitosan is a very versatile and widely-used biopolymer primarily because of its availability and biocompatibility. The most common application of chitosan in cancer imaging broadly lies in three different imaging systems, *i.e.*, a fluorescent-based optical imaging system (confocal microscopy, near-infrared imaging/NIR), X-ray-based system (mainly CT scan), and MRI.

17.8.1 Chitosan Nanomaterials for Fluorescence-based Imaging of Cancer

The use of chitosan nanomaterials in fluorescent-based imaging is very common nowadays. One can find a considerable number of promising research publications on fluorescence-based cancer cell imaging by chitosan nanomaterials. One of the most common uses of chitosan is as the carrier for the fluorescence dye into the cells.^{174–178} Zhu *et al.* used LDL (low-density lipoprotein)-coupled *N*-succinyl chitosan nanoparticles labeled with Cy7 fluorescence dye to image H22 liver tumors *in vivo*.¹⁷⁴ LDL was the key molecule here. It worked as the targeting moiety for the LDL receptor present in the liver cells. Because of the precise targeting, the detection of the tumor inside the mice could be made possible. Another study by Na *et al.* demonstrated the liver targeting and fluorescent imaging ability of glycol chitosan-5 β -cholanic acid nanoparticle Cy5.5 fluorescence dye.¹⁷⁵ More interestingly, they have also claimed that chitosan nanoparticles are more likely to be taken up by the acidic cancer cells than the other nanoparticles like polystyrene nanoparticles and phosphocholine nanoparticles. They have also shown that the *in vivo* NIR signal

in chitosan nanoparticles was intact for up to one full day. To support the NIR data optical tomography and correlative light-electronic microscopy analysis were also performed, and the results were highly satisfactory. Another study by the same research group tested glycol chitosan nanoparticles on various tumor models to support the claim that chitosan nanoparticles are superior to other nanoparticles when targeting cancer cells, making them an excellent candidate for imaging.¹⁷⁶ Chitosan-modified quantum dots have also been used for cancer imaging with great success.^{177,178} Mansur *et al.* used chitosan stabilized self-fluorescing cadmium sulfide (CdS) quantum dots for imaging osteosarcoma.¹⁷⁷ RGD peptide was conjugated with chitosan to target $\alpha_v\beta_3$ integrin receptors, which are overexpressed in cancer cells. Wang *et al.* fabricated an injectable chitosan–carbon dot hybrid nanogel.¹⁷⁸ They have used it for confocal as well as NIR imaging of 4T1 cells *in vitro* and 4T1 tumor bearing mice *in vivo*, respectively. The carbon dots in the nanogel were responsible for the bright and stable fluorescence, which resulted in the detection of the tumor inside the mice with reasonably good accuracy.

Chitosan-based nanoprobe and sensors are also being used for cancer imaging.^{179–183} Ma *et al.* fabricated carboxymethyl chitosan-coated CdTe (cadmium telluride) quantum dot (CMC-CdTe QDs)-based intracellular probes on sensing Zn^{2+} in prostate cancer cells (PC-3M).¹⁷⁹ The CMC-CdTe QDs were found to have a green-yellow fluorescence increases with increasing concentrations of CMC. Mannosylated chitosan–zinc nanoprobe have been reported for use in detecting oral cancer epithelial cells (KB cells).¹⁸¹ The nanoprobe has been tested against KB cells, where the mannose receptors are over-expressed, and L929, where no mannose receptor is expressed. The KB cell surface was much more crowded with a high amount of nanoprobe than the L929 cells, which shows minimal non-specific binding. Gold nanocluster-based probes coated with chitosan-*N*-acetyl-l-cysteine capable of strong fluorescence at 680 nm were developed by Duan *et al.* for versatile cell imaging applications.¹⁸⁰ Upon testing against HeLa cells, the nanoprobe showed stable and robust fluorescence, with very low sensitivity toward cancer cell content (H_2O_2 and protease). The study further described the versatility of *N*-acetyl-l-cysteine as it contains free amino and carboxyl groups, which could easily be modified with other functional groups or targeting moieties like biotin or folic acid. Zhu *et al.* used α -hederin chitosan NPs tagged with Cy7, which is a near-infrared (NIR) fluorophore, to image cancer cells *in vivo*.¹⁸² α -Hederin was used as an anti-cancer agent because of its significant cytotoxic effect toward cancer

cells by inducing apoptosis.^{184–186} CD147 antibodies, which are highly specific to the cancer cells HepG2 (human hepatoma), were further conjugated to the nanoprobe, resulting in the accumulation of the nanocarrier in target cells at higher concentrations leading to the very good and resolved NIR image. A fluoroimmunoassay-based hydrophilic nanoprobe was fabricated from CdS quantum dots and CD20 polyclonal antibody-modified chitosan.¹⁸³ This system was successfully tested for fluorescence imaging against non-Hodgkin lymphoma (NHL) cells.

17.8.2 Chitosan Nanomaterials for Enhancement of CT and MRI Resolution

Chitosan-based nanomaterials are being used for the improvement of the resolution of CT and MRI images.^{187–191} Lim *et al.* have developed *N*-naphthyl-*O*-dimethylmaleoyl chitosan-based magnetic nanoparticles (NChitosan-DMNPs), which were used as pH-sensitive drug release and MRI contrast-enhancing agents as well.¹⁸⁷ The magnetic core of the nanomaterial enables MR imaging, which was quite evident from the *T2*-weighted images of tumor-bearing mice. It showed a better resolved MR image post-injection of the nanomaterials than pre-injection. Another glycol-chitosan-based pH-responsive nanomaterial was developed by Nwe *et al.* that can act as a contrasting agent for MR imaging.¹⁸⁸ Gadolinium, which is a widely used contrasting agent, was attached to the nanomaterial. They were able to create a significant amount of contrast enhancement in *T1*-weighted MRI with a dose that is as low as 1/3 of the recommended dose of gadolinium. Another chitosan-based system, along with gadolinium, was employed for MR imaging of cancer tissue.¹⁸⁹ Arachidyl chitosan was used to make the nanomaterial more positively-charged to facilitate its binding with the negatively-charged cancer cell membrane. *In vitro* paramagnetism was evaluated by 4.7 T MRI, and the resulting *T1*-weighted images showed increasing MR signals with increasing concentrations of gadolinium. Lee *et al.* used chitosan–linoleic acid nanoparticles with a superparamagnetic iron oxide nanocrystal (SPIONs) core to image liver cancer.¹⁹⁰ The localization of nanoparticles into the liver was facilitated by the linoleic acid conjugated with the chitosan, and the SPION played the role of MRI contrasting agent.

17.8.3 Chitosan Nanomaterials for Multimodal Imaging

All the detection techniques, be it optical imaging or X-ray based detection techniques, have their pros and cons. So, scientists have developed many chitosan-based materials capable of performing multimodal imaging,¹⁹²⁻¹⁹⁹ and the glycol-chitosan-based material is one of the most common of all. Key *et al.* reported a glycol-chitosan-based nanomaterial for multimodal imaging of cancer.¹⁹² Glycol chitosan has been conjugated with 5 β -cholic acid and Cy 5.5 and used it to synthesize nanoparticles with SPIO embedded inside. They were able to perform NIRF and MRI imaging of tumors in live mice, but it is essential to mention that a higher magnetic field (3 T) is needed for detection through MRI. The same research group has published another article later, where they have added a bladder cancer-targeting peptide (CSNRDARRC) to the nanomaterial and made it capable of targeting bladder cancer specifically, which further improved the contrast of the image.¹⁹³ Na *et al.* have reported DOTA-modified gadolinium-encapsulated glycol-chitosan 5 β -cholic acid nanoparticles to image liver cancer and subcutaneous tumors in live mice.¹⁹⁴ Like the previous study, Cy 5.5 acted as the NIRF dye, which provided a 2.4-fold higher signal in cancer tissue than the normal cells, resulting in a clear image of cancer tumors (see [Figure 17.5A](#)). On the other hand, gadolinium, already known as a popular contrasting agent, was used in the nanomaterial for MRI imaging of the tumor. The nanocarrier showed superior contrasting efficacy over the commercial contrasting agent DOTAREM[®] because of the improved tumor accumulation of the nanomaterial by the enhanced permeation and retention (EPR) effect (see [Figure 17.5B](#)). Similar glycol-chitosan-based nanoparticles and a couple of important modifications were also employed for NIRF/PET imaging by Lee *et al.*¹⁹⁵ Cy 5.5 was used for NIRF imaging as described in previous studies, but they conjugated ⁶⁴Cu radiolabeled DOTA complexes with chitosan nanoparticles for PET signaling. To target cancer in A549 tumor-bearing mice, an activatable matrix metalloproteinase (MMP)-sensitive peptide (GPLGVRGKGG) was chemically conjugated *via* bio-orthogonal click chemistry as the MMP is found to be overexpressed in cancer cells. Other important works have been published, where similar chitosan-based nanostructures were used with Cy 5.5 as the NIRF imaging agent and iron oxide nanocore as the MRI contrasting agent by Lin *et al.* and by Lee *et al.*^{196,197} Both the nanomaterials produced good NIRF and MRI images of cancer tissue in live tumor-bearing mice, but the cancer-targeting mechanism was different in both cases; FA was used to target cancer in the former one, whereas the EPR effect has been utilized in the latter case.

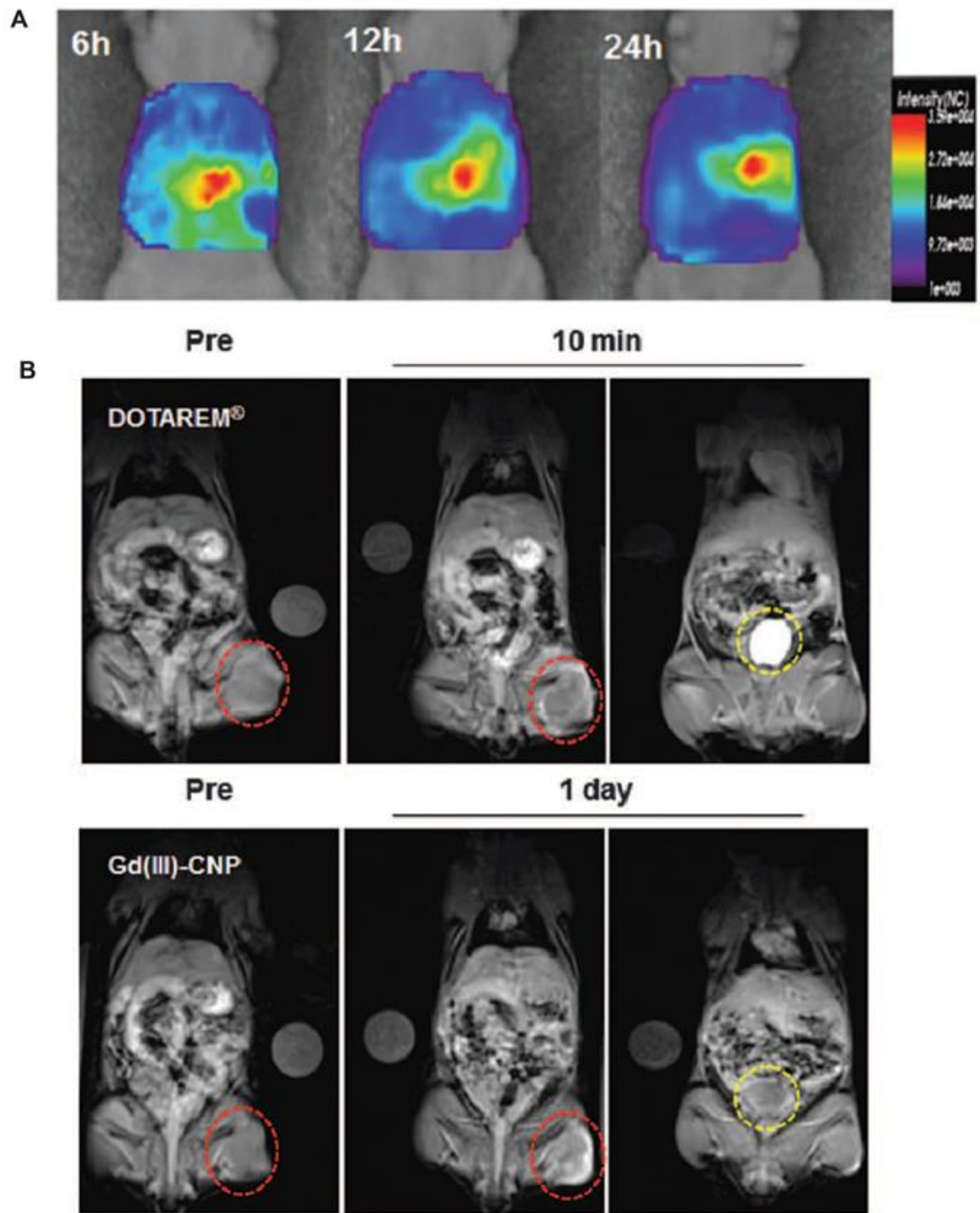


Figure 17.5 (A) Whole-body NIRF images of the liver tumor-bearing mice 6 h, 12 h, and 24 h post-injection of Cy 5.5-labeled Gd(iii)–chitosan nanoparticles. (B) *T1*-Weighted MR images of subcutaneous tumor-bearing mice after injection of Gd(iii)–chitosan nanoparticles and

DOTAREM[®] (red circle: tumor site; yellow circle: bladder). Adapted from ref. 194 with permission from the Royal Society of Chemistry.¹⁹⁴

17.9 Conclusion

Due to the versatile nature of chitosan, it finds wide application in cancer drug delivery and biomedical imaging. Chitosan possesses unique physicochemical properties that make it a very significant candidate for pharmaceutical applications. It can be formulated into various types of materials such as nanoparticles, microparticles, nanocomposites, hydrogels, and conjugates, *etc.*, that can be used as effective anti-cancer drug-delivering cargo. Different types of anti-cancer drugs can be introduced into the body *via* various routes using chitosan-based materials. Due to the presence of amino and carboxyl groups on its backbone, many modifications can be done to it, which can impart various properties to it, including cancer cell targeting and stimuli-responsive behavior. Not only as an anti-cancer agent, chitosan also found an important place in the field of cancer imaging. The research works we have discussed here clearly indicate that the present techniques are not enough to detect cancer with great accuracy. But chitosan-based nanomaterials brought hope in the field of cancer detection. In this chapter, the nanomaterials we have discussed show their potential in upgrading the existing techniques. As a result, cancer detection will become more accessible, leading to ease of treatment and low mortality.

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Chapter 18

Polysaccharide-based Scaffolds for Tissue Engineering Applications

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18.1 Introduction

The mainstay of tissue engineering are cells, scaffolds, and growth stimulating signals. Scaffolds are materials that contribute to the structural support and tissue development in general. Scaffolds can be said to be very similar or analogous to the extracellular matrix. Scaffolds usually have porosity which serves as a medium for cell adhesion and proliferation. The materials used for tissue engineering scaffold formulation should be

analogous to the extracellular matrix composition which is made up of collagen, gelatin, and glycosaminoglycan (GAG). Mimicking of the native tissue is a herculean task owing to its complex composition and dynamic nature. A comparison of synthetic and natural polymers shows that the design space available with the bio-based polymers is quite large and tremendous.¹

Polysaccharides are widely available in nature and in various natural sources such as alginate, agar, algae, galactans, carrageenan, cellulose, pectin, guar gum, xanthan gum, dextran, gellan gum, pullulan, chitin, chitosan, chondroitin, hyaluronic acid, and bacterial cellulose. They are classified based on their electric charges, the negatively-charged polysaccharides are hyaluronic acid, heparin, pectin, and alginate, the positively-charged polysaccharide is chitosan, and the electroneutral polysaccharides are dextran, agarose, starch, *etc.*,² These polysaccharides are considered as excellent biomaterials which offer great potential because of their biocompatibility, hydrophilicity, solubility, biodegradability, and functionality. There are few limitations of the polysaccharides in which most of them are soluble in water, and some of them can't be employed in biomedical applications. Therefore this drawback can be overcome by combining the natural polysaccharides with other natural or polymeric materials through chemical modifications. The polysaccharide structure has hydroxyl, amino, and carboxyl groups and this property is very useful in the chemical modifications.³

Biopolymers are gaining importance in tissue engineering as they have quite similar groups, analogous to the extracellular matrix composition. Biopolymer-based scaffolds can support cell attachment, cell growth, proliferation, *etc.* which are main requisites for tissue engineering so they have excellent biocompatibility. The biopolymeric scaffolds based on alginate, dextran, chitosan, *etc.* have excellent processability, mechanical properties, and wettability making them ideal candidates in the field of tissue regeneration. There are many studies reporting that these polysaccharides play an important role as biomaterials in repairing bone, cartilage, cardiac, and skin tissues in the tissue engineering fields.⁴ This chapter deals with the properties of polysaccharides, mechanisms, biological properties, and polysaccharide-based scaffolds in the tissue

engineering fields. In the conclusions and outlooks section we briefly discuss the use of polysaccharides in fabricating biomaterials and intend to convey the viewpoints and impediments for the prospective research and development of polysaccharide-based materials. In addition, this chapter outlines the exceptional applications of polysaccharides as a biomaterial in the fabrication of scaffolds.

18.2 Chemical Modifications of Polysaccharides

Natural polysaccharides are associated with a few drawbacks, some of which are the high hydrophilicity of cellulose, poor solubility of chitosan, and the fact that some of them can't be used in biomedical applications.⁵ Hence, these drawbacks prevent the wide usage of polysaccharides in various biomedical fields. As a result, the chemical modifications of polysaccharides provide a smart solution for improving the chemical and biological properties of the materials. These polysaccharides can be modified easily owing to its multifunctional chains and it is very important to know the concepts and methods of chemical modifications for modifying polysaccharides. There are several methods of modifying polysaccharides through chemical means. The usual methodologies for the modifications are graft polymerization, carboxymethylation, etherification, esterification, oxidation, sulfation, and so on. In this chapter, the very important and typical chemical modification processes are briefly explained regarding the general protocols of chemical modification of polysaccharides.⁶

18.3 Graft Polymerization

This is one of the important modification techniques and is mostly used to modify the surface properties of materials for different applications. The graft polymerization technique is one of the easiest methods used to improve the biocompatibility of synthetic polymers. Generally the free radical polymerization method is one of the commonly used techniques for the grafting of polymeric materials. This method involves the generation of free radicals with the help of initiators and monomers on the biopolymer backbone.⁷ In a study they have developed different kinds of grafted

polysaccharides with polyacrylonitrile using a ceric saccharide redox process. This free radical polymerization process certainly forms homopolymers, lacking regulation of the polymer structure, and it is difficult to remove them. Thus controlled radical polymerization methods like reversible addition fragmentation chain transfer polymerization and atom transfer radical polymerization have been widely adopted because of their degree of controllability. The research shows that controlled radical polymerization methods are an effective modification process to attain the functionalization of polymers.⁸ An example of this kind is that nanocellulose was effectively modified with vinyl pyridine through atom transfer radical polymerization and as a result it forms self-healing hydrogels used for biomedical applications.⁹

18.4 Sulfation

Sulfation is an effective technique for changing the chemical and biological properties of polysaccharides. It is reported that the hydroxyl groups of monosaccharides at the C6 linkages are very active and they have hydrogen bonding interactions. These are some of the agents used for the sulfation of polysaccharides: sulfur trioxide, chlorosulfonic acid, pyridine, sulfuric acid, and sulfur trioxide dimethylacetamide. Amongst all these reagents, the chlorosulfonic acid–pyridine method is one of the most used agents because they are easy to process and give a high yield.¹⁰ Another interesting example reported that the sulfated polysaccharides are most widely used for antitumor activities. When there is alteration in the molecular weight, chain length, or charges of the chemical properties of the biomaterial it results in the increase or change of biological activity.¹¹ In a study, the *in vitro* coagulation assay showed that the sulfated alginate reports a very high hemocompatibility. Moreover, the sulfated polysaccharides greatly depend upon the degree of substitution and this characteristic is more responsible for the biocompatibility. Another study showed that the sulfated polysaccharides possessing a different degree of substitution result in the highest immunomodulatory activity.¹²

18.5 Carboxymethylation

This modification method is a widely used technique in which one of the hydrogen atoms from the hydroxyl group is replaced by a carboxymethyl group. The polysaccharides are dissolved in sodium hydroxide then reacted with chloroacetic acid and the pH was adjusted to obtain the purified carboxymethylated derivative. This results in an enhanced activity or it provides a new bioactivity compared with the unmodified polysaccharides.¹³ In a reported study they have processed a carboxymethylated polysaccharide from *Lachnum* exopolysaccharides which showed higher antioxidant activity and in addition exhibited high anticancer activity.^{14,15}

18.6 Esterification

There are many commercially available modified polysaccharides, esters of cellulose, starch, and hyaluronic acid (HA), because of their versatility with valuable properties. Many of the studies used esterified HA as a scaffold by esterifying the carboxyl groups with the repeating units of glucuronic acid in the polymer chain. In this technique the ester groups are introduced by an esterification process in which the properties of the esterified polysaccharides are affected by the substitution, its degree, position, and their hydrophilic nature, and negative charges are also reduced.¹⁶ It is also reported that the degree of esterification of hyaluronic acid is inversely proportional to its solubility in water. There are several options available to perform the esterification process and they are dialkylcarbodiimide, iminium chlorides, and carbonyldiimidazole substituted for the transesterification and ring opening esterification techniques.^{17,18}

18.7 Alginate and Dextran-based Scaffolds

Alginate and dextran are polysaccharides which have exceptional biocompatibility and biodegradability with multitude of applications in the biomedical field. These materials can be processed as three-dimensional scaffolds such as hydrogels, fibers, foams, microspheres, microcapsules, and sponges. Alginates are used as carriers in drug delivery systems and tissue engineering. Moreover, they can be surface-modified through

physical and chemical reactions to obtain different types of materials with several structures, properties, functions, and categories.¹⁹ By modification of the structure through chemical or physical crosslinking by immobilizing the ligands with peptide and sugar molecules, the specific properties of a material can be achieved such as good biodegradability, gel property, affinity with cells, and mechanical strength. This part focuses on alginate, dextran, and its derivatives for use in biomedical fields such as in bone tissue repair, regeneration, drug delivery, and wound healing.^{20,21}

18.7.1 Hydrogels Based on Alginate and Dextran

These hydrogels are cross-linked three-dimensional networks consisting of hydrophilic polymers which have a high water-content and when the cells are inserted into them, they become swollen and transport the nutrients into the cells and the waste out of them. They are water-soluble and thicken in neutral conditions which makes them suitable for *in situ* hydrogel formation. Based on their gelation mechanisms, they can be classified into covalent and physical gelation and there are many methods engaged in the formulation of hydrogels. These are ionic interactions, cross-linking reactions, thermal gelation, free radical polymerization, and click reactions. These types of hydrogels show pH responsive behaviors which have a high swelling rate at increased pH because of the presence of ionic carboxy groups. Ionic cross-linking is the widely used method for preparing hydrogels using ionic crosslinking agents. Mostly, Fe^{2+} , Ba^{2+} , Ca^{2+} , and Mg^{2+} are used for cross-linking ionically with materials. Another method of preparing injectable hydrogels is through phase transition because the gelation is achieved above the lower critical solution temperature as the temperature is increased. Through the addition of PNIPAAm in its backbone, the thermal sensitivity of the hydrogel can be attained (Figure 18.1).²²

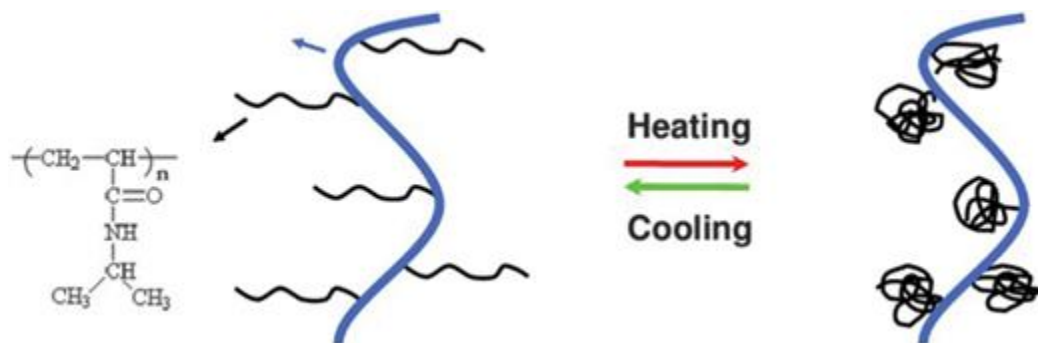


Figure 18.1 Temperature dependent behavior of PNIPAAm–alginate hydrogels.

Moreover, the alginate and dextran hydrogels are prepared by using cell cross-linking with the help of ligands such as arginine, glycine, and aspartic acid which are sequenced on the hydrogels for cell adhesion. This method particularly helps in forming network structures through the ligands and the specific receptors on the cell surface which have excellent biocompatibility (Figure 18.2).²³

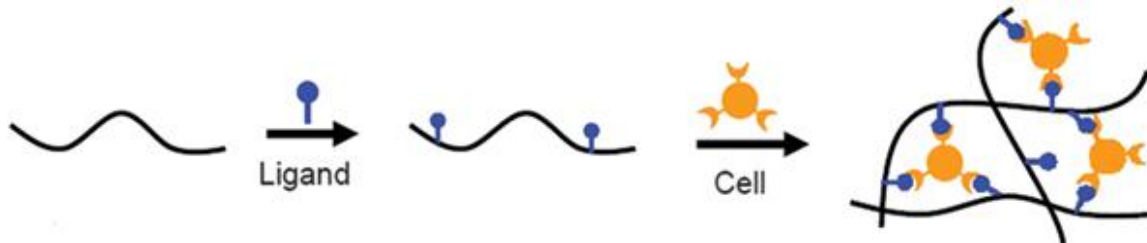


Figure 18.2 Cell-crosslinked network formation of ligand modified alginate/dextran.

Free radical polymerization is the process of altering a linear polymer into 3-dimensional polymer networks along with the initiators which can have direct contact with the drugs and cells at a particular pH and temperature. In this method, it is easy to incorporate the chemical techniques to incorporate in the hydrogels by mixing with the macromers and copolymerizing them. An example shows that free radical polymerization of methacrylate alginate with the C–C groups formulates hydrogels and this can be used as a cell delivery vehicle for tissue engineering/tissue regeneration. Through photo polymerization reactions,

the methacrylated alginate was covalently crosslinked with the help of redox reaction using ammonium persulphate (APS) and tetramethylethylenediamine (TMED) which forms a hydrogel at body temperature (Figure 18.3). This particular scaffold was used to encapsulate human ASCs and chondrocytes and when it was injected into the body, it formed a crosslinked alginate gel which was very useful for tissue engineering aspects.^{24,25}

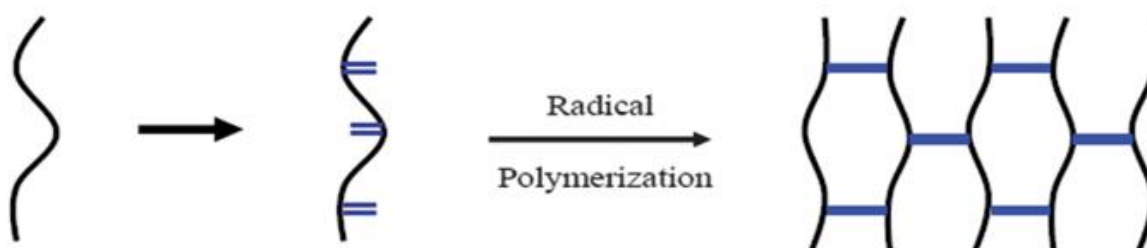


Figure 18.3 Preparation of methacrylated alginate/dextran and photo crosslinking of methacrylated alginate/dextran.

Click chemistry uses click reactions with the cycloaddition of 1, 3 dipolar copper with azides and alkynes. The main limiting factor of these metal-mediated click reactions is due to the toxicity of transition metals and therefore metal-free alternatives were developed recently. They are called metal-free click chemistry and involve the conjugation of cells useful for the tissue engineering aspects. The alginate–dextran composite hydrogel has been developed based on the concept of the click conjugation reaction which is highly biocompatible and biodegradable. Based on the Schiff's reaction with the oxidized alginate/dextran and amino groups of gelatin C–C bonds crosslinked with amino groups through Schiff base linkage are formed. The metal-free conjugated complexes are very appropriate for a broader range of biodegradable, biocompatible scaffolds without any chemical crosslinking agents (Figure 18.4).^{26,27}

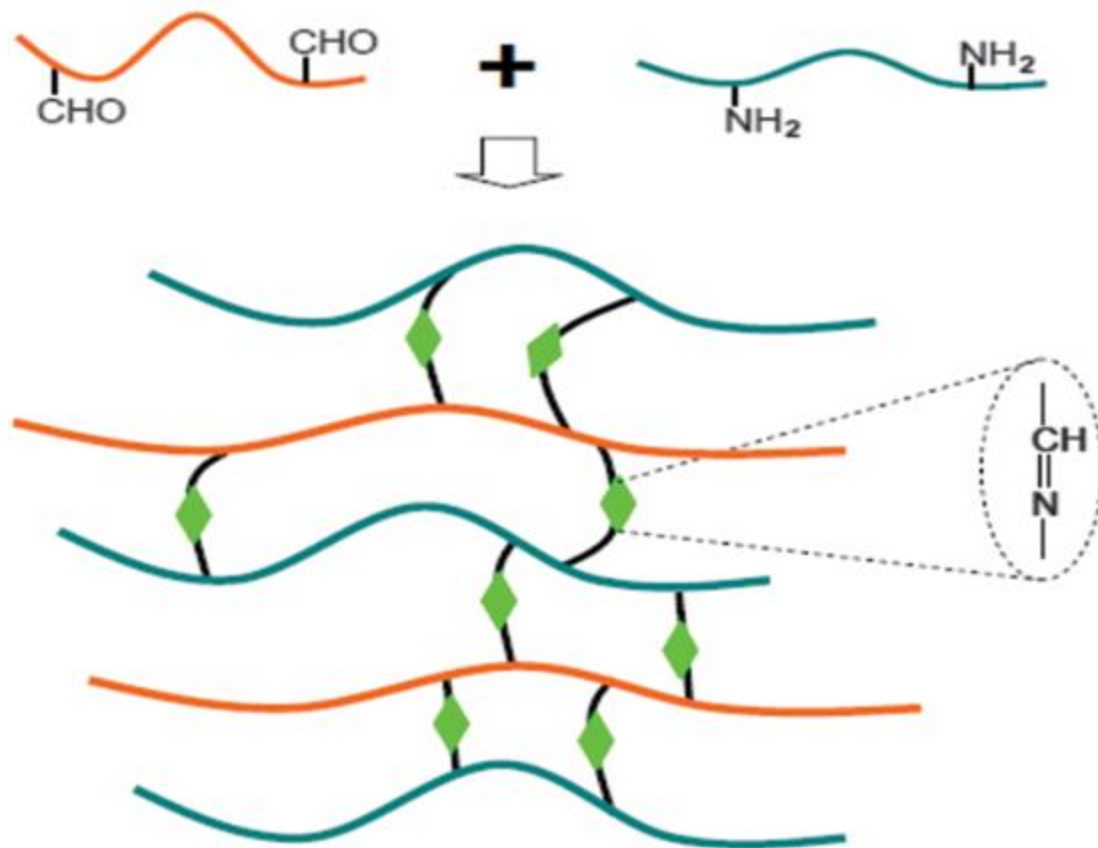


Figure 18.4 Alginate–dextran composite hydrogel *via* the Schiff-base reaction.

18.7.2 Microspheres Based on Alginate and Dextran

Microsphere formulations are used to deliver cells, growth factors, proteins, genes, and drugs in biomedical applications. Gel and solid microspheres are formed through certain reactions, for example, the gel spheres are formulated using ionic crosslinking whereas the solid spheres are formulated using an emulsion solvent evaporation system. They are highly biocompatible and biodegradable which is useful in tissue engineering, drug delivery, and other biomedical applications.

Gel spheres are the ones which deliver the cells, bioactive molecules, and growth factors, and they encapsulate all these materials which are delivered to a localized area and released in a sustained manner. They will encapsulate the bioactive molecules, cytokines, small proteins, and other bioactive molecules which provides a protective shell/covering for localized delivery.^{28,29} With the presence of ionic Ca²⁺, the alginate and dextran gel

spheres were used for the controlled delivery of bioactive molecules. The bioactive molecules are mixed with the alginate/dextran solution and the gel microspheres are prepared in a CaCl_2 solution with constant stirring. The size of the spheres are 200–500 μm and they are homogenously dispersed in the gel spheres (Figure 18.5). The consistent pore size and morphology can be achieved through the microfluidic device. The gel spheres are crosslinked as 3D scaffolds with interconnecting pores.³⁰

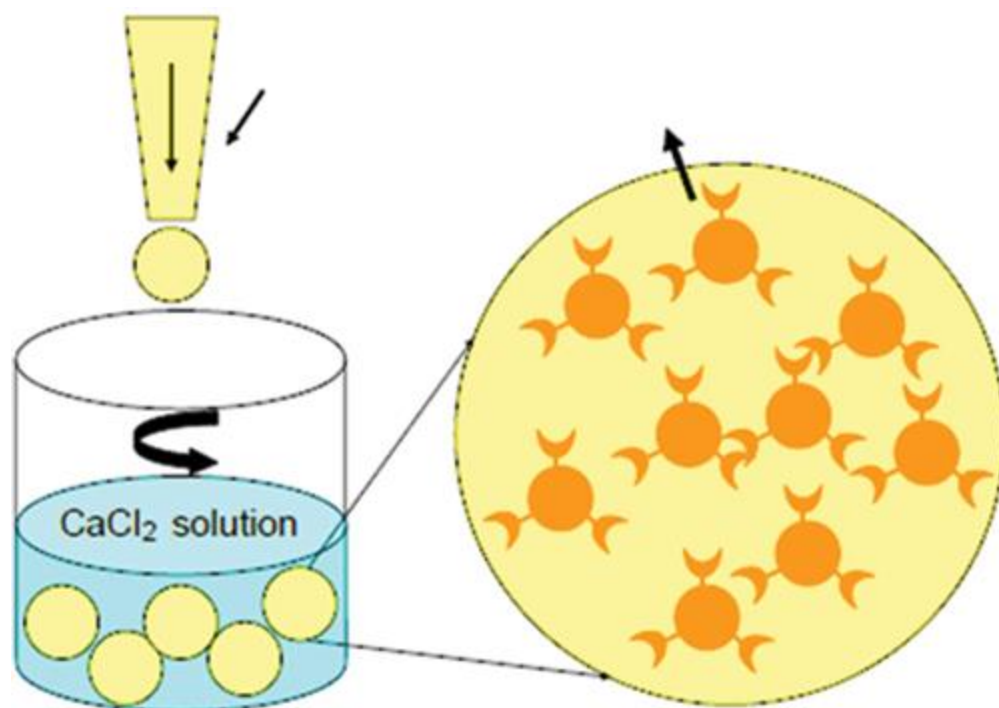


Figure 18.5 Procedure for alginate/dextran gel-spheres in containing cells.

Solid spheres are the ones that have been extensively used as carriers for drugs, growth factors, and other bioactive molecules. Usually chitosan, PLA, PLGA, dextran, and alginates are used to prepare microspheres and nanoparticles. The drugs are mixed with the alginate/dextran solution and it is emulsified under sonication and the drug-loaded microspheres are formulated by adding the bioactive molecules drop-wise to the organic emulsion under constant stirring (Figure 18.6). They protect the drugs from degradation and also deliver the drug very effectively as a carrier for many biomedical applications. The surface of the spheres/nanoparticles are

hydrophobic so they are taken more rapidly from circulation and get cleared from the blood through the mononuclear phagocyte system (MPS).^{31,32}

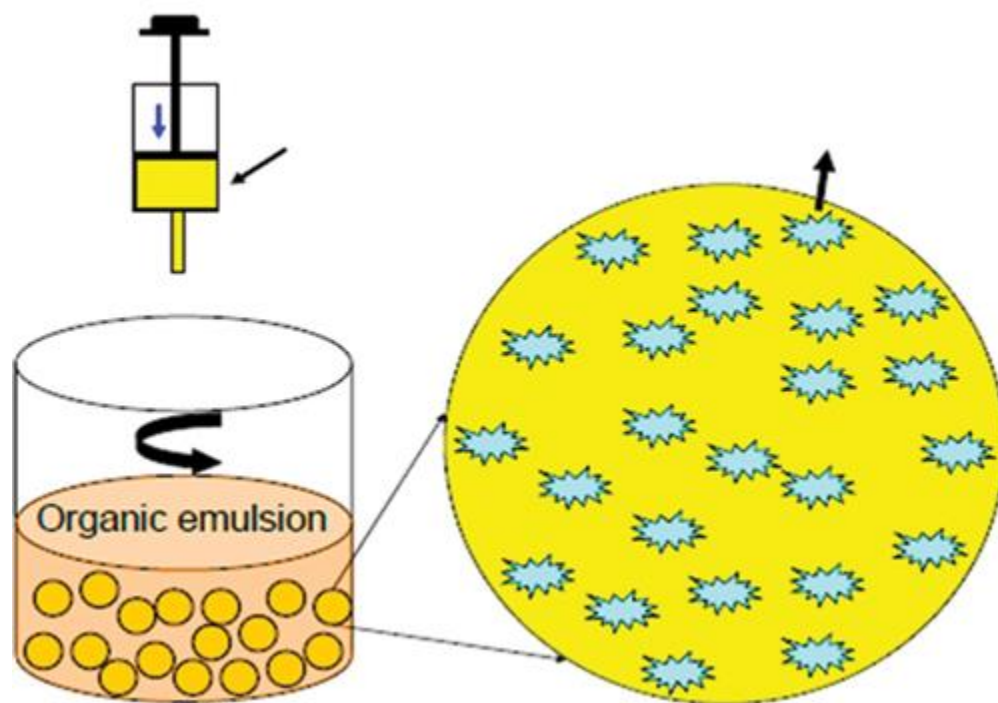


Figure 18.6 Procedure for alginate/dextran solid-spheres in loading drugs.

18.7.3 Scaffolds Based on Alginate and Dextran

The traditional methods for manufacturing porous biopolymer scaffolds are gas foaming, solvent casting, phase separation, freeze-drying, and particulate leaching. Compared to all other methods, the freeze-drying method is one of the easiest and most effective ways to fabricate the scaffolds. The dextran/alginate-based scaffolds or sponges which have an interconnected porous structure with an expected shape can be easily achieved through a simple freeze-drying method. The rate of biodegradation and the strength of the freeze-dried scaffolds mostly depend upon their polymers and other crosslinking agents. The mechanical strength of the scaffolds depends on the pore size, porosity, and the orientation. However, the pore size in the porous scaffolds may not be uniform and the components present in the scaffolds affect the biodegradation rates.^{33,34} The

electrospun nanofibers are widely used for tissue engineering applications and the main role of the cell scaffold is to mimic the extra cellular molecules (ECM) and gives support to the developing tissues. The ideal scaffold is one that has similar characteristics to those of the ECM with respect to composition as well physico-chemical properties. The alginate/dextran-based nanofiber matrix is very similar to that of non-woven ECM proteins. Electrospinning is a very facile method to manufacture nanofibrous mats which is used for a wide range of applications. The main important features of the electrospun nanofibrous mats such as pore size and fiber diameter can be designed using the solution properties such as viscosity and concentration and also tailored using the flow rate and electric field. The thickness of the mat is also affected by the distance/size of the collector plate and the total mass deposited over the plate. The alginate/dextran-based mats are promising nanofibrous scaffolds fabricated for wide usage as tissue scaffolds with certain pore sizes and diameters. Moreover, the nanofiber mats usually have a flat surface and less thickness, and very densely packed fibers are widely used for tissue engineering applications where they have less cell infiltration. These nanofibrous scaffolds can be tailored by various approaches to meet their specific applications.³⁵

18.8 Applications of Alginate/Dextran Scaffolds

18.8.1 Wound Healing

For centuries, dressings have been applied to open wounds to prevent the wounds from injury and bacterial infection. Previously, gauze was mainly used as a dressing material as it has a high absorbent capacity and is cost effective, however during peeling off from the wound it creates secondary injuries. So, these days novel high quality wound dressings are widely fabricated to create a good environment to enhance the healing of wounds. There are many dressings and sponges, gels, occlusive, and semi-occlusive dressings have been reported and alginate/dextran materials have been used in various wound dressings. The alginate/dextran-based dressing materials are sponges, hydrogels, and electrospun mats and they are highly favorable

substrates for wound healing. The advantages of these materials are their gel forming ability to absorb wound exudates, great hemostatic capability, conformability, mild antiseptic properties, nontoxicity, biodegradability, water vapor transmission rate, and biocompatibility.^{36,37} The marketed alginate dressing Kaltostat is able to improve the wound healing by stimulating the monocytes to produce high levels of interleukin-6 and TNF- α (tumor necrosis factor α). Pro inflammatory factors such as the release of cytokines are important for enhanced wound healing.³⁸ Alginates have endotoxins in them and because of their presence they have high bioactivity at wound sites. In a previous study gelatin, alginate, and borax were used in scaffolds which showed a hemostatic effect and promoted wound healing with the antiseptic potential of borax used as a wound healing material.³⁹ In another study the authors revealed that dextran hydrogels improved the healing of wounds by promoting the regeneration of the dermis and also facilitating cell infiltration. By promoting angiogenic cells into healing wounds, the neovascularization process occurs by day 7 which increases the blood flow. And by day 21, the burn wound area forms mature epithelial cells and collagen formation with improved hair follicles. After the 5th week, the dextran hydrogel scaffolds lead to the growth of new hairs and a mature skin structure.⁴⁰

18.8.2 Cartilage Repair

There is huge importance of tissue-engineered cartilage for clinical use because the degenerative and traumatic lesions of the articular cartilage are the prominent aspects of disability. There are millions of people suffering from osteoarthritis and tissue engineering methods are employed to enhance regeneration. Injectable implants also play a major role in cartilage repair because they offer immediate stiffness and strength to the cartilage tissue. In the case of the alginate hydrogels, their physical and chemical properties can be matched with those of the articular cartilage for regeneration of cartilages. In the case of alginate-based hydrogels, solid and gel microspheres were used for articular regeneration. Some of the studies have reported that the alginate-based microspheres and the hydrogels were used for the release of growth factors to the cartilage tissue in a controlled manner. In one study, they prepared alginate microspheres using a

microfluidic device which were effective with chondrocytes for the regeneration. Moreover, the animal studies also proved that the structures of cartilage were formed within four weeks of implantation. Another study proved the co-encapsulation of TGF, MSCs, and HA along with the microspheres, and these hydrogels result in repairing the cartilage. The ideal scaffold has the capability of loading and releasing the biomolecules which has improved mechanical strength to promote the growth of cells and cell differentiation. In one study, they used dextran hydrogels encapsulated with the endothelial cells to make them form different vascular structures. This type of hydrogel supports the presence of endothelial cells, fibroblasts, muscle cells, hepatocytes, and cardiomyocytes.^{41,42} After implantation, these hydrogels take part with the host cells through micro-vascular structures and they get degraded with the tissue infiltrates. The dextran hydrogels containing VEGF promote cellular infiltration, tissue growth, and rapid biodegradation. Moreover, the encapsulation of VEGF along with the IGF-1, SDF-1 induces larger blood vessels and the combination of all the growth factors produces a synergistic effect through which it increases the size and amount of neovascular structures. The hematoxylin, eosin, and smooth muscle staining of the tissues showed that RBCs were stored which indicates the blood vessels are functional.⁴³

18.8.3 Drug Delivery

The carriers for drug delivery applications have been playing a major role for delivering the drugs, large molecules, biomacromolecules, proteins, and genes in the localized or in the targeted action. Alginates and dextran molecules are widely used as a carrier for many drugs, bioactive substances, proteins, and cells because of its biodegradability and biocompatibility. There are hydrogels, colloidal particles, and complexes that are widely used. Alginate/dextran hydrogels, porous scaffolds, microcapsules, and microspheres are used for controlled drug delivery, biosensors, micro reactors, and tissue engineering, and these can also be formulated into a layer-by-layer assembly with the polyelectrolytes.⁴⁴ In addition, they also have a good amount of loading and controlled release profile. The derivatives of dextran-based molecules were formulated using different functional groups which include allyl isocyanate (Dex-AI), chloroacetic

acid (Dex-AC), maleic anhydride (Dex-AM), and ethylamine (Dex-AE). For the fabrication of dextran hydrogels, polyethylene glycol is used as a cross-linking agent and based on tuning the properties, they do have different release profiles and degradation rates. Based on the different physico-chemical properties they are influenced by some factors such as swelling, crosslinking density, biocompatibility, and biodegradability. By combining amine groups with the dextran in hydrogel formulation, they do have better biocompatibility and release properties. The different functional groups influence the basic properties of the hydrogel network and amine groups for the delivery of drugs and other biomedical applications.⁴⁵ A study which was shown to fabricate biopolymer microcapsules were deposited onto the CaCO_3 particles showing excellent biocompatibility by means of electrostatic interaction. The different degradation studies also showed that by immersing the microcapsules with different pH values for estimating the number of layers which is involved in stabilizing the microcapsules. Moreover, the addition of PEG to the dextran and alginate leads to protection against the acidic environment whereas the coating layers influence the swelling behavior, degradation, and the compatibility of the microcapsules.⁴⁶

18.9 Chitosan-based Scaffolds

Chitosan (CS) is a natural polycationic linear polysaccharide. It is a copolymer of α -glucosamine and *N*-acetyl- α -glucosamine units linked with linear β -(1 $\rightarrow$ 4) glycosidic linkages. However, α -glucosamine is the predominant repeating unit. It is easily soluble in mild aqueous acidic conditions in a pH range of 5–6. CS is derived from the deacetylation process of chitin under alkaline conditions. In this process, acetyl groups of chitins are eliminated and amino groups are introduced. Chitin is a structural element found in the exoskeleton of crustaceans. The polycationic nature is conferred through the protonation of amino groups under acidic conditions. Polymeric gels can be easily formed utilizing the positive charge of CS through participation in electrostatic interaction with a negatively-charged molecule. The molecular weight of CS varies between 300 kDa to 1000 kDa, and the higher the molecular weight the higher the

viscosity. CS exhibits pseudoplastic or shear-thinning behavior, an essential property for the development of inks for bioprinting applications. The degree of deacetylation (DD) also varies for CS between 50–95%. It has been reported that DD impacts degradation behavior and cell attachment for CS-based scaffolds. Highly deacetylated CS having DD >85% is suitable for cell attachment and exhibits a slow degradation profile. CS membranes are very stiff and brittle and are used for tissue engineering purposes. In order to optimize the mechanical resistance and elasticity, crosslinking agents with two functional groups are often used for making bridges between the polymer chains. One such promising crosslinking agent is glutaraldehyde; the aldehyde groups react with the amine groups of CS and form imine bonds.⁴⁷

CS exhibits biocompatibility, biodegradability, and antimicrobial properties, and shows no toxicity. The biodegradability of CS is inversely related to the polymer's crystallinity, which is related to DD. Lysozyme is the primary enzyme responsible for the hydrolysis of the β -(1 $\rightarrow$ 4) glycosidic linkages of CS. Oligosaccharides or monosaccharides are the degradation products of CS. The effectiveness of lysozyme is restricted when the DD of CS is below 30%. The biocompatibility of CS is conferred by its minimal foreign body response and fibrous encapsulation when implanted *in vivo*. CS also helps in modulating functions of inflammatory cells and promotes granulation of tissues, thereby accelerating the wound healing abilities. The positive charge of CS helps it to bind with the negatively-charged blood cells, making it a promising candidate for wound dressing applications. Similarly, the positive charge of CS is believed to disrupt the anions in bacterial cell walls. The biosynthesis and mass transport across the cell walls is inhibited, and CS exhibits an antimicrobial property. CS has a natural chelating ability that binds with endotoxins of Gram negative bacteria and reduces toxicity. The functional groups (hydroxyl and amine) of CS enable it to be used as a drug carrier. The hydrophilic nature of CS facilitates cell adhesion and proliferation.⁴⁷

18.9.1 Chitosan Derivatives for Tissue Engineering

Several chemical modifications have been carried out on CS structures to improve their physical and chemical properties concerning tissue

engineering applications. The majority of chemical modifications involve the primary hydroxyl group at the C₆ position and the amino group at the C₂ position. The secondary hydroxyl group at the C₃ position cannot rotate easily at their spatial conformation, and due to the high steric hindrance, the secondary hydroxyl group cannot react efficiently. The introduction of aliphatic or aromatic acyl groups in the CS structure terminates inter- and intra-molecular hydrogen bonds, reduces crystallinity, and improves water solubility. The acylation reaction at the C₂ position with the –NH₂ group forms an amide, and the reaction is known as *N*-acylation, while the reaction at the C₆ position with the –OH group forms an ester and the reaction is known as *O*-acylation. *N*-Acetylated CS is primarily used in biomedical engineering as they are carriers for sustained release of drugs, enhancing biocompatibility and anti-coagulability. Improvement of water solubility can also be achieved by the introduction of an alkyl chain in the molecular structure. The alkyl group is hydrophobic, and hence the length of the alkyl chain controls CS derivatives' solubility. Strong nucleophilic lone pair electrons of C₂–NH₂ groups facilitate the *N*-alkylation reaction. The carboxylated derivative of CS is widely in wound healing applications. Under the alkaline conditions, the reactivity of C₆–OH is greater than C₂–NH₂, and carboxylation mainly occurs at the C₆ position. However, when the degree of substitution is greater than or equal to 1, carboxylation occurs simultaneously at both sites. Grafting of hydrophilic quaternary ammonium groups also improves water solubility. Apart from biocompatibility, quaternary ammonium salts also have innate mucoadhesiveness and an ability to bind with epithelial cells. Sulfated CS has structural similarity to heparin and offers anti-coagulability. The primary amino groups of CS can react with the thiol group containing coupling agents, and the thiolated CS derivatives enhance mucosal adhesion. Covalent bonds are formed between free thiol groups and cysteine-containing glycoproteins in mucus. Grafting copolymerization is another method to impart additional characteristics to the CS. Polyethylene glycol (PEG), polylactic-co-glycolic acid (PLGA), and gelatin are some of the copolymers grafted onto CS. PEG-grafted CS offers solubility over a wide range of pH.^{48,49}

18.9.2 Chitosan-based Scaffold Fabrication Methods

Phase separation and lyophilization is a commonly used scaffold fabrication method in which CS solution is poured into a mold and subsequently is subjected to controlled freezing. Ice crystals are formed in the CS acetate salts, which sublimates during lyophilization leaving behind a porous scaffold. The interfacial tension at the scaffold-air tension often collapses and forms a non-porous layer at the film's surface. The advancement of the phase separation technique includes incorporating porogen in the CS solution, which is eventually removed in the solvent leaching process before the lyophilization step. This method provides the flexibility of controlling the pore size. However, the particulate leaching method is time-consuming and offers limited pore interconnectivity. In an alternative approach, gas bubbles can be introduced to the system to obtain the porous structure. The gas bubbles can be incorporated by mixing foaming or blowing agent with a prepolymer, which generates gas bubbles upon chemical degradation. Apart from that, exposing CS solution with highly pressurized subcritical or supercritical gas followed by depressurization led to the formation of bubbles. The porous scaffold can also be formed by removing the lyophilization step after phase separation. In this approach, the gelation of CS solution is achieved by exposing the phase-separated CS to sodium hydroxide/ethanol at -20° before the drying step.⁵⁰ Hence, in this process, lyophilization is not required. A schematic representation of the process mentioned above is depicted in [Figure 18.7](#).

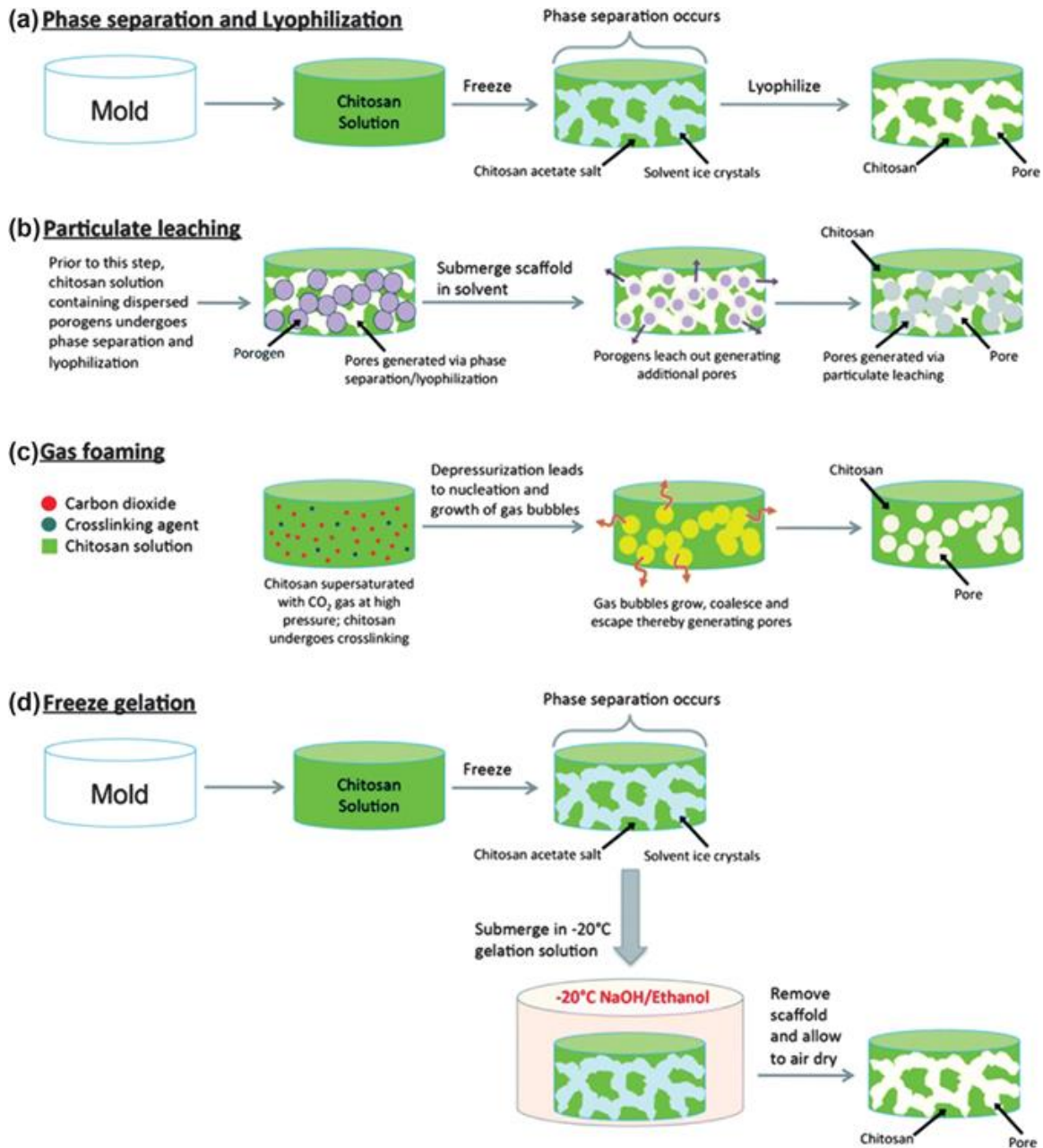


Figure 18.7 CS based scaffold fabrication methods. (a) Phase separation and lyophilization technique, (b) particulate leaching technique, (c) gas foaming technique, and (d) freeze gelation technique.⁵⁰

Polyelectrolyte complexes are formed by mixing the positively-charged CS solution with negatively-charged alginate, gelatin, or hydroxyapatite. These polyelectrolyte complexes can be used as ink for the fabrication of scaffolds in the bioprinting method.⁵¹ CS scaffolds can also be formed by

fusing or sintering CS microparticles or microspheres in a densely packed mold. CS microspheres are formed by ionotropic gelation or *via* a crosslinking method. The use of mild aqueous acid can expedite the particle aggregation process. The scaffolds formed in this technique have higher density and superior mechanical properties than scaffolds prepared through the phase separation and lyophilization technique. However, the scaffold drastically lacks porosity.⁵⁰ Nanofibrous CS scaffolds are formed utilizing an electrospinning technique in which CS is dissolved in a volatile solvent. It is challenging to form pure CS-based scaffolds in the electrospinning process due to the highly polycationic nature of CS. Hence, it is often blended with other electro-spinnable biopolymers.⁵² The polymer solution is ejected slowly through a syringe attached to a blunt needle in the electrospinning process. An electric field is applied between the needle tip and a grounded collector plate. A high voltage at the needle tip charges the polymer solution and deforms it into a conical shape. When the charged polymer overcomes the surface tension of the solution, a straight polymer jet is ejected and stretched. The volatile solvent is removed, and the polymer jet experiences instability, and randomly, it is deposited on the grounded collector plate.⁵³

18.9.3 Chitosan in Tissue Applications

Modification of surface roughness helps in overall cell adhesion and proliferation in wound healing applications.⁵⁴ CS helps in hemostasis, inflammation, proliferation, and tissue remodeling. The hemostatic activity of CS depends on its molecular weight and DD. The amine group of CS affects blood coagulation activity. It has been found that a moderate DD around ~66% favors interaction with blood components with its mesh-like structure. However, CS with higher DD offers strong hydrogen bonds with a highly crystalline structure that limits its interaction with red blood cells. Higher molecular weight also influences the coagulation ability. The CS dimension plays a crucial role against inflammation. Macro-sized CS-based scaffolds are promising against inflammation over nano or micro-CS particles. CS stimulates the proliferation ability of dermal fibroblasts. For bone and cartilage tissue engineering, scaffolds with improved mechanical properties are required. CS imparts suitable mechanical properties with

interconnected pores apart from its biocompatibility. CS can induce apatite formation, a requirement for biomineralization in bone tissue engineering. It assists in the accumulation of calcium/phosphate ions and promotes osseointegration. The structural similarity of CS with sulfated glycosaminoglycans provides a natural environment for chondrocyte proliferation, chondrogenic differentiation, reduces chondrocyte inflammation, and helps in cartilage tissue engineering. CS also forms electrostatic interactions with collagen. CS has widely been used as a carrier for drug delivery applications. Self-assembled polymeric micelles are one of the suitable drug delivery systems using CS derivatives.⁵⁵ In regenerative medicine, CS has widely been used for soft nerve tissues. In neuroregeneration, transplantation of stem cells on chitosan-based carriers has some possible outcomes.⁴⁹ CS also has wide applications in blood vessel tissue engineering and liver tissue engineering.⁴⁷

18.10 HA-based Brain Tissue Engineering

GAG is the principal component of the brain and so HA-based scaffolds can be exploited in brain tissue engineering. Wang *et al.* in 2008 prepared a HA, collagen-based 3D-porous composite scaffold by a freeze-drying method and tried to determine its suitability in brain tissue engineering. SEM and light microscopy confirmed the sheet structures of the scaffold with perforations quite similar to the honeycomb structure. This composite remained in the hydrated state as HA has a very high water-uptake capacity. It is said that HA can exhibit 60-fold increments in weight after hydration. The hydrated composite scaffold showed similar compressive modulus like brain tissues. A cell culture study showed that neural stem cells assembled to the neurosphere which showed neurite outgrowth and axon extension forming a neural network gradually. A similar pattern was even observed on the 3-D HA-based composite scaffold. All *in vitro* and physicochemical studies confirmed the suitability of HA in brain tissue engineering.

18.10.1 HA-based Adipose Tissue Engineering

Preadipocytes, stem-cell derived precursor cells can differentiate into various types of cells and use of these cells for soft tissue engineering

perhaps can bring massive success in the treatment of plastic and reconstructive surgery. Due to the high proliferation and differentiation capacity, a suitable porous-structured 3D scaffold supporting its growth is needed. HYAFF[®]11 a biopolymer-based scaffold with a pore diameter of 400 microns facilitated the seeding of Preadipocytes.⁵⁶ HYAFF[®]11 is actually a modified linear derivative of HA whereas the carboxylic group of the glucuronic acid monomer was esterified by the benzyl group and it was further processed by submerging in HA solution, followed by a freeze-drying process. SEM images confirmed the pore size. The hydrophilicity of the HYAFF[®] scaffold significantly enhanced the HA coating proved by its turning into a blue colored scaffold after quick absorption of bromphenol blue solution. Incubation of the scaffold with preadipocytes under different culture conditions showed good attachment, distribution, and proliferation of adipocytes over days by MTT assay. So, *in vitro* study proved its success that it can be used as a supportive structure for tissue engineering *via* helping cell development stages which lead to subsequent tissue repairing processes. Grafting of a scaffold on mice didn't show any infection or mortality of the animal. Grafting inside the mouse started to form thin blood vessels in all layers of the scaffold which was confirmed by microscopical observation of the scaffold and texture of the scaffold after vascularization changed too. Due to cell growth and all the relevant changes, the weight of the scaffold increased with time. The scaffold didn't shrink over time as all the pores were occupied with new developed cells. Though the scaffold acted as a good autologous carrier for cell attachment and proliferation, HYAFF[®]11 scaffolds with such a large diameter were unable to stimulate differentiation of progenitor cells to adipose tissue. Seeding of carriers with pre-differentiated preadipocytes were suggested. In another study carried out by another group it was investigated that instead of an appropriate scaffold structure, architecture, pore diameter, mechanical property and rheology, *in vivo* study of the scaffold produced a new fibrous tissue. Adipose tissue was not generated on the scaffold but different kinds of cells other than adipocytes were grown on the scaffold. Thus the HA-based scaffold showed its utility in soft tissue engineering, but it needs further optimization for being used in adipose tissue engineering from a biological point of view.⁵⁷ Collagen–HA-based scaffolds were also tried for adipose

tissue engineering. In another study by Davidenko *et al.* they showed differentiation of preadipocytes and mammary stromal tissue development by *in vitro* study while maintaining all the physical supports needed for tissue engineering. The scaffold was fabricated by a freeze-drying method.⁵⁸

18.10.2HA-based Cartilage Tissue Engineering

If cartilage is damaged, generally its repairing process is very slow due to the avascular and aneural nature of cartilage. Tissue engineering may bring hope for repairing of cartilage among other medicines and different clinical approaches. Hyaluronic acid/chitosan coacervate-based scaffolds were exploited for cartilage tissue engineering for its biocompatibility and biodegradability. SEM confirmed the presence of many small pores inside of bigger pores confirming the porous structure. The lyophilization process was adapted for drying of coacervate and during drying, the pores were shrunk a bit due to water removal but still a porous structure was maintained. A biodegradation study showed that the scaffold was able to hold its shape intact almost for 120 days. Rat bone marrow stem cells (rBMSCs) were added to the coacervate and incubated for up to 21 days. Cell proliferation was found well indicating the viability of cells over time on the scaffold. Observation of the morphology of the cells over the scaffold was assisted by a confocal microscope *via* a staining method. The cell morphology remained the same even after 21 days of incubation. Though *in vitro* chondrogenic differentiation and *in vivo* experiments were not performed in this study, three-dimensional (3D) scaffolds of higher water-content and appropriate viscoelastic properties showed the ability to support cell interaction, viability, and proliferation of cells needed for cartilage tissue engineering.⁵⁹

18.10.3HA-based Nerve Tissue Engineering

A freeform fabrication technique was employed using a digital micro mirror-array device (DMD) for fabrication of HA-based scaffolds for neural tissue engineering. Before fabrication of the HA scaffold it was modified with photopolymerizable glycidyl methacrylate. UV light exposure through micro mirrors only for 30 s was able to create hexagonal, circular, and

square patterned scaffolds of different pore sizes. The prepared scaffold was washed with phosphate buffer saline to remove oligomers synthesized during the photo polymerization process. The scaffold remained biologically active even after a UV assisted method by showing its binding activity with biotinylated hyaluronic acid binding protein (HAbp). The biodegradability of the HA-based scaffold in the presence of biodegradable enzyme hyaluronidase was detected through longer exposure to UV slowed down the degradation process due to enhanced cross-linking density within the polymeric chains. Grafting of cell adhesion protein laminin on the HA scaffold aided in Schwann cell adhesion and cells remained viable for 36 h. A DMD method was able to introduce multiple biomolecules such as growth factors spatially within 3D scaffolds. These outcomes show the DMD fabricated HA scaffold may bring a revolutionary approach in nerve tissue engineering.⁶⁰

18.10.4HA-based Skin Tissue Engineering

Chitosan, gelatin, and HA cross-linked composite scaffolds were fabricated by a freeze-drying method and sterilized by gamma irradiation. The scaffold of the heterogeneous structure and varying pore sizes appeared in the SEM images. Due to a high retention of water by the composite scaffold, it holds its good shape and good size during cell culture and engineering of tissue. Good attachment, growth, and proliferation of cells were found on the composite scaffold. Historical appearance of fibroblast and keratinocytes over scaffolds has been shown by secretion of collagen and keratin respectively. The SEM study confirmed the necessary morphological changes of fibroblasts and keratinocytes within the scaffold. Artificial skin tissue regeneration can be possible based on a HA-based scaffold.⁶¹

18.10.5HA-based Other Soft Tissue Engineering

Poly-lactic acid (PLA)-based tissue engineering scaffolds in spite of the biodegradability and biocompatibility lack the wettability and reactive functional groups needed for tissue engineering. Atmospheric pressure plasma assisted HA immobilization on the PLA-based electrospun scaffold which might bring an improvement in its performance.⁶² Plasma-assisted immobilization of HA on the scaffold didn't cause any damage of PLA

electrospun fiber shown by SEM and full wetting of the scaffold was confirmed. The cell viability on the HA-coated PLA scaffold has shown its biocompatibility.

The success of any scaffold depends on how it reacts with macrophages during the implantation phase and in this investigation elevated levels of biomarkers (interleukins, cytokine) were found using an enzyme-linked immunosorbent assay (ELISA). All these observations showed the scaffold's reaction with macrophages came with preliminary mediation of inflammation, wound healing, and secretion of matrix metalloproteinase (MMPs) degrading extracellular matrix protein. But optimization of the HA amount to 0.1 wt% concentration for coating on PLA proved it to act as the most biocompatible scaffold and this scaffold didn't stimulate pro-inflammatory responses. Culturing of human umbilical vein endothelial cells (HUVECs) on this particular scaffold showed HUVEC tube formation and showed its pro-angiogenic activity. These kinds of scaffolds can be useful for tissue engineering of skin, blood vessel, nerve, bone, *etc.*

18.11 Future Prospects and Conclusions

The need for other solutions that can meet the demand for replacement organs will continue to drive advances in tissue-engineering biomaterials for the future. The biologically-derived polymers offer multiple advantages in the bioengineering perspective. The biopolymers presented in this review provide tissue engineers with a range of properties that can mimic existing native tissues. The excellent biocompatibility of polysaccharide hydrogels, such as alginate and chitosan, are suited for use in soft-tissue applications or even non-load-bearing tissue types such as bone or cartilage. The similarity of these biopolymers which helps in seeding of cells that at a later stage can proliferate into the tissue types.

Last but not least the usage of biopolymer-based matrices for biomaterials and tissue engineering will be a thrust area as many common factors are matched with the existing extra cellular matrix. There are many challenges that excite engineers in developing fundamental methods and applications in material formulation that help in the development of more-complex cellular structures.

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